

3 July 1980

Venice, Energy, Brandt and politicians

Meetings of political leaders such as that which ended in Venice on 23 June are always risky for the participants. The chief risk is simply that they fail to agree on whatever it is they have chosen to talk about, the final communique says little more than that a meeting has taken place and doubts are cast on the value of high-level consultation as such. The seven Western politicians gathered in Venice have avoided that risk. Their parting communique was at once more detailed and more constructive than for many a year. It remains to be seen, however, whether they will be able to avoid the second hazard that faces summit-goers — that having agreed on something they go back home and discover there is nothing they can do to put flesh on the bones of what they have agreed. The chances of that happening are, unfortunately, all too real. It has, after all, taken a full seven years for the heads of government to come to the belated recognition, embodied in their communique from Venice, that something must be done about the price of oil.

The argument is familiar. Increasing OPEC prices have brought about the curious blend of inflation and deflation from which the industrialized nations of the West are suffering. It has also jeopardized the future of the developing countries, which must keep on borrowing from abroad in circumstances which must throw doubt on their capacity ever to repay their debts, and which certainly diminish the chances that they will be able again to move forward socially and economically. So, the Venice communique has it, the industrialized West must diminish its own dependence on OPEC oil, must aim at making its own industry more productive and must not be seduced by the blandishments of those who argue, on narrow national grounds, for more constraints on international trade. In short, the major industrialized states appear belatedly to have recognized where their true economic interests lie, and have also made more than a passing acknowledgement of the still more serious problems of the developing countries, most recently and cogently described by the Brandt Commission (*Nature*, 21 February). So far, so good.

But why have the industrialized states not had the gumption to say all this before? That is a natural question to ask. In other less exalted contexts, in the Council of the International Energy Agency and the OECD for example, bits and pieces of the problem have been tackled. Why have the heads of government been so slow off the mark? The explication is not wholly creditable. It is only in the past year or so that all the governments represented in Venice have found themselves companions in misfortune. Both West Germany and Japan have found it possible for most of the past seven years to import oil and to pay the bills for it like men, without having to resort to their currency printing presses. It is also true that, during most of that time, several of the governments represented at Venice have been following economic policies that would have allowed inflation to bounce along at an alarming rate even if the price of oil had remained just a few dollars a barrel. The American, British and Italian governments have been the chief offenders. Readers of their communique from Venice must be forgiven the suspicion that some of the fine words are not backed by sufficient resolution. Some OPEC governments will complain, with justice, that the price of oil is being made a scapegoat for deeper ills.

The same kind of scepticism will attach to what the communique says about the steps the governments have agreed upon to lessen their dependence on OPEC oil. As these things go, the Venice document is unusually specific, replete with words such as "base-load". Plainly the world's leaders have been got at

by their technical people, which no doubt is why the civil servants of the West are convinced that Venice was a "success". And, of course, it is sensible enough that the West should forswear oil-burning power stations, thereby avoiding extra demand for imported oil for the following quarter of a century. But did anybody in Venice think of asking Mrs Thatcher, the British Prime Minister, why the British nationalized utility, the Central Electricity Generating Board, is still trying to complete the oil-fired power station on the Isle of Grain (on the south bank of the Thames estuary) when the workforce of ladders (men who wrap insulation around pipes) has refused to work except at salaries which offend other workers (and their unions) on the site? The Venice declaration is also full of promises that there will be incentives, "where necessary" including fiscal incentives, to encourage the exploration for oil. Why, then, is the deregulation of United States oil prices not yet complete? Why has the British government just put up the tax on oil production in the North Sea? Why has the search for a way of taxing the "windfall profits" of the oil companies become the equivalent in the 1980s of the spate of legislation against "monopolies" in the 1920s?

The politicians at Venice can only blame themselves if some of their other specific declarations on energy policy provoke the same kind of disbelief. For the medium term, the declaration says, nuclear power, coal and conservation must bear the burden of helping to slacken external oil dependence. This, of course, has been clear for a decade. The trouble is that the governments at Venice have also known this to be the truth, but have mostly failed to make progress. The British government has only just emerged from what has been essentially a moratorium on decisions about nuclear power. (One ironical consequence is that British Nuclear Fuels Limited, which had wanted to build a prototype of a plant to vitrify nuclear waste as long ago as 1974, is now having to think of using a French process instead — see page 5.) In the United States, the Nuclear Regulatory Commission limps along with a stand-by chairman whose successor, soon to be appointed, is likely to be replaced again when the Administration changes. United States coal production, which President Nixon promised would reach 800 million tons a year at least by 1980, remains only 600 million tons a year, largely because of the endless troubles about strip-mining projects. In Britain, the National Coal Board still waits to know (after a public inquiry on the subject) whether it will be allowed to exploit a profitable reserve of coal in the Vale of Belvoir. But these, alas, are only some of the ways in which performance has fallen behind precept in energy policy. What is to be done?

Some intolerant souls, offended by this contrast, have been saying that the Venice governments should take the bull by the horns and ram what are undoubtedly sensible policies down the throats of their people. Quite apart from being a recipe for electoral disaster, such a course would risk throwing away the baby with the bathwater. The reason why it is important that the West should be freed from its dependence on OPEC oil is that money inflation is destroying valuable social institutions (educational systems, health care services, charitable institutions) while the accompanying deflation is hampering innovation. It would not however be acceptable that for the sake of being liberated from these economic shackles, people in supposedly democratic countries should be told from on high what to do and then required to put up with the consequences. Would the game then be worth the candle? Do not even self-styled and usually unqualified ecologists deserve a hearing? On nuclear power, there



are special reasons why the professional community has a special role to play in ridding the nuclear wrangle — it is not an honest debate — of mumbo jumbo. Here and elsewhere, however, the responsibility for getting things done lies also with governments. If higher (or even higher) energy taxes are thought necessary, it is for them to persuade their voters that the benefits will be worthwhile — the chance to remain citizens of progressive yet dynamic communities. If strip mines, or ordinary coal mines, or nuclear power plants, are necessary, governments have a duty to make sure that their people know what are the stakes — the avoidance of economic debacle, to put it at its mildest; at the other end of the spectrum, the avoidance of the much more serious trouble there would be bound to be if, say, Saudi Arabia were unable to produce 11 million barrels of oil each single day.

Telling it like it is is unfortunately unfashionable. Signing communiques is easier. So too is making promises for the long term that become confused in voters' minds with solutions to immediate problems. President Carter's promise two years ago that 20 per cent of American energy production would come from the sun by the end of the century is of this kind. For the industrialized West, the question is not that of what the energy mix will be in the year 2000 but how to get from here to there. Implicitly, that is what the heads of government at Venice said. They had better now tell their people even if, as a result, they suffer the traditional fate of the bearers of bad tidings. They

might make their message more palatable by seeking more imaginative ways out of their present fix — by counterbalancing the rigged OPEC market in oil with their own free market (*Nature*, 12 May). This week, for what it is worth, the spot price at Rotterdam has fallen below that charged by some OPEC countries, which can only suggest that the price would fall even more quickly if the market were assured of a regular share of the West's own oil production.

Happily, however, Venice was not entirely a recitation of familiar platitudes. Habitually, the industrialized West has collectively been shy of saying much about its obligation to the developing countries of the world for fear of giving too many hostages to fortune. At Venice, they broke cautiously with precedent and even went as far as to "welcome" the report of the Brandt Commission, promising to "study" its recommendations. They also undertook not to organize their policies on international trade in ways that would be harmful to the non-oil developing countries. That is as it should be, although it is doubtful whether the Venice signatories appreciate the magnitude of their undertaking; it implies nothing less than recreation of their industrial base at a time of perhaps indefinite recession. Such magnanimity will not come easily to the unemployed shoeworkers of New England or the unemployed workers of the British steel and motor car industries. Nor is there much hope that the industrialized West can share this burden with other states.

Why bother with school examinations?

LIKE Stratford-upon-Avon, the Changing of the Guard and the game of cricket, the British examination system is one of those characteristically British phenomena which the rest of the world knows it will never understand. Increasingly, the British are in the same plight. Now that the examinations season is at an end, when only the most diligent of those who have survived their recent trauma have begun preparing for the next, and when many of those responsible for assessing the gigantic paper output of the examination system have withdrawn temporarily from the real world, it may be safe to raise the question whether the system as a whole is worth preserving. The answer is not simple. Plainly the system cannot be entirely swept away but it is equally unthinkable that it should be preserved in its present form.

The rules of the game are worth a little effort to understand, if only for the light that understanding may throw on what must surely be one of the most pathetic of human frailties — the belief that ordinary men and women in some stratum of the academic profession can reduce any other person's quality to a number of some kind, usually between zero and 100. One of the curiosities of the recent proliferation of examinations in Britain is that in the 1950s the country was riven into the camps of those who passionately held that it is possible by means of an examination to tell at the age of eleven whether a child should have an academic or grammar school education, and the camp of those who considered the eleven-plus examination to be unjust. In the end, the abolitionists more or less won the day, comprehensive schools were invented and for a time preoccupation with school examinations seemed to have been stilled.

Alas, the wish to examine and to be examined has since reasserted itself with a vengeance. Increasing numbers of no doubt fond parents now subject their sons and daughters, often at the age of seven or eight, to the competitive examinations put up as hurdles by the fee-paying schools, knowing that their offspring will thereafter be graded once a year either by means of a number or, more elegantly, by a letter from the Greek alphabet, with pluses or minuses appended. For most British children, the first external yardstick comes at about 16, the age of O (which stands for "Ordinary") level, or alternatively of "CSE" (which stands for Certificate of Secondary Education). The word that echoes around the corridors of secondary education is that you cannot get a job unless you do reasonably well in one or other.

In ancient days (but even now in Scotland) there used to be an examination called "School Certificate" devised at least in part to

meet the entrance requirements of universities. Since entry to universities was in question, universities came to dominate the committees which administered these examinations. Knowing that not every university could run its own examinations board, the universities grouped themselves into consortia, of which there at present nine, and which now administer the O-level examinations and the A (for "Advanced") equivalents, literally used by most universities as a way of telling which students should be given a chance to study something serious.

The problems of the sixteen-plus examination are unfortunately minor compared with those which crop up two years later. The rules of the game, in Britain, are that those wishing to find themselves a place in a university are required not merely to jump the hurdle of the O-level examinations but of A-level as well. They are, for practical purposes, required to elect two years in advance for what they will take an interest in at university, and then to prove (with the help of the school-teachers who happen to be around) that they can perform. Many youngsters rebel at choosing, but eventually are forced to toe the line. Others, no doubt, give up, or worse still find they have chosen wrongly. In all secondary schools, teaching A-level students is regarded as the pinnacle of academic life (and in many schools it is quite remarkable how successfully the image of specialist can be created at sixteen or seventeen).

If the charge against this part of the British educational system were merely that it is farcical, it might be allowed to continue. The truth is that it is actively harmful. It ensures that young people are too narrowly prepared for the academic life, and frequently have been forced at an early age to choose the wrong disciplines to follow. In justice, and in the national interest of the British, it would be better if A-level were to be abolished. Since, however, that will not happen overnight, it would serve the same purpose if A-level were to be subverted. The way in which this might be done is easy to identify. If only universities were once again to take responsibility for their student entry, and were to select say ten per cent of intending students by some quite different method — a scholastic aptitude test, an interview or some other indicator of performance — the result would be that lots of bright young people would be found to prefer that route. If selection for the people not taking A-level were a year earlier than for the rest, but if university courses were a year longer for the early ten per cent, there would at least be a chance to tell which way was the best. Is it not time that this was tried?

New Basel director changes direction

The Basel Institute for Immunology, founded in 1971 by Hoffman-La Roche, the Swiss pharmaceutical manufacturer, is soon to have a new director, the 44-year-old chemist and geneticist Fritz Melchers. Dr Melchers, already a member of the institute, will take up his appointment with effect from 1 October.

Melchers will succeed Dr Niels Jerne, the first director, who has helped to give the institute its now outstanding reputation in basic immunology. During his tenure of the post, Jerne has also fought strongly for the independence of the institute from the company which finances it, insisting on the freedom of the members of the institute (who have no other titles) to pursue whatever research appears appropriate, to publish in the scientific literature and to pay no more attention than they wish to the research interests of their parent company.

Inevitably there is now some concern that the old arm's length policy will be changed. Melchers agreed on the telephone last week that his policy may be "more friendly" than that of Dr Jerne. The new director says, however, that he intends to maintain the role of the institute as a centre for fundamental research in immunology, cell biology and molecular genetics. Applied work will have no place at the institute, he says, and researchers will be completely free to publish their results.

Melchers says that he would not have taken the job unless these conditions had been satisfied. The future of the institute has also been underwritten by the company "for a considerable period". The implication is that the integrity of the research programme will not be compromised by short-term money problems.

The institute has a small tenured staff, but provides facilities for a much larger number of visiting scientists. It was originally set up by Roche for a limited period of ten years, although Jerne has said that Roche told him it would keep the institute going "so long as the Western world did not change drastically". Jerne took this to mean that the laboratory would continue at least as long as the world took Valium, one of the company's most successful products.

Some change in the relationship between the institute and the company is nevertheless on the cards. Melchers said last week, for example, that the role of the scientific board of the institute, as well as the mechanism for providing scientific advice to Roche, are under consideration. The function of the board has been to advise the company on the scientific work of the institute and "all matters relevant thereto". Melchers said last week that "we want a board of independent scientists who will help in the exchange of ideas with the firm while maintaining the scientific integrity of the institute". The company, he said, "has a growing interest in

molecular biology and its applications", and correctly feels that the subject should be part of its research effort. Roche, said Melchers, spends a lot of money on the institute and deserves some return from it.

This does not imply that the institute will become a wing of Hoffman-La Roche research, however, Melchers says, but rather that communications between the institute and the company should be

improved. He cites the setting up of a hybridoma research group at Roche with the institute's help as an example of the improved communications he has in mind.

One present member of the board, Avron Mitchison of University College, London, greeted Melcher's appointment with delight. "He is an able scientist, decisive, and can deal with the company."

Robert Walgate

Sitting-in in Spanish labs

DEMONSTRATIONS of scientists are uncommon events, even in Spain. On 27 May, however, more than five hundred scientists of the research centres of the CSIC (*Consejo Superior de Investigaciones Científicas*) marched through the streets of Madrid to the Ministry of Universities and Research in one of the most spectacular events in three weeks of protest by Spanish researchers.

Hopes of a change in government policy towards science were aroused by the establishment of a democratic regime in Spain. The experience of the past three years has, however, been disappointing, despite some beneficial changes in the structure of the CSIC and universities.

It now seems that the creation of the Ministry for Universities and Research has not brought the benefits expected. In fact, the 1980 budget for research has increased by only 7.2 per cent, a figure far below the inflation rate. As a consequence, financial support for research has decreased in real terms compared even with the past level.

Thus the budget for one of the main sources of grants, *Fondo Nacional de Ayuda a la Investigación* (FNAI), was withheld for more than a year and afterwards fixed at only 2,500 million pesetas (\$35 million), one-eighth of the applications.

This may be too late to avoid the interruption of the research in certain cases. At the same time, another important source of support for research — the programme for predoctoral fellowships — has been cut by a half. This means that an average university may receive fewer than 30 fellowships — and this at a time when unemployment among young graduates is reaching dramatic proportions.

To add to these uncertainties, the abolition of the CSIC has been considered within the government and publicly discussed. A new Universities Bill (*Ley de Autonomía Universitaria*) has been presented unchanged to parliament in spite of criticism from many sections of the scientific and university communities.

Against this background, the staff of the Institute of Astrophysics and the Experimental Station of El Zaidin in Granada recently decided to sit in as a protest against "the chaotic situation existing in the CSIC". A manifesto was issued

denouncing the worsening condition of Spanish research and proclaiming that the trouble stemmed from the absence of a coherent science policy, the inadequacy of the funding for research and the low salaries and lack of promotion for scientists and technicians. Shortly afterwards, a meeting was held in Madrid by scientists and technicians and that was followed by the demonstration ending at the Ministry for Universities and Research with the reception of a delegation by the minister.

On 2 June, the minister attended the first session in Madrid of a National Congress on Science Policy after a request by the Association of Research Personnel, which consists mainly of CSIC workers. In his speech, the minister acknowledged the absence of a science policy in Spain and conceded that the present situation was caused by the low level of funding.

The day the Congress on Science Policy ended, the Ministry of Universities and Research issued a statement promising that the CSIC will not be dismantled but radically reorganized. In another statement, the ministry announced the publication of a White Paper on research and a Three-Year Plan for research. The publication of a White Paper was said to be imminent in August 1979, and a Three-Year Plan has already been promised for more than three years.

Pedro Puigdomenech

Correction

The report on 26 June of the views of the Astronomy II Committee of the Science Research Council appears to be substantially incorrect. The committee did not decide to ignore SRC policy on the appointment of postdoctoral fellows; did not offer advice to senior researchers on how best to deal with short-term appointments; and did not empower the Royal Astronomical Society to act on its behalf. The position appears to be that the Astronomy II committee did discuss the problems of short-term appointments but postponed a full-scale discussion until a meeting in October. We regret the error.

Editor, *Nature*

High-energy physics

LEP leaps ahead

THE European Centre for Nuclear Research (CERN) last week made a firm proposal to its 12 member states for a new accelerator to be built in the 1980s. The machine, called LEP, is a 30 km circumference ring for accelerating, storing and colliding very high energy beams of electrons. The proposal already bears the mark of CERN's firebrand director-general-designate, German Professor Herwig Schopper: LEP is now cheaper than in any former design — 900 million Swiss francs (£235 million) compared with 1100 million Swiss francs (£280 million).

The scientific case for LEP is considered by high-energy physicists to be outstanding, better than that for any accelerator since the early 1950s, when the Lawrence cyclotron was built to create what was then thought to be the quantum of the nuclear force-field — the pion. LEP, it is hoped, would produce the intermediate vector bosons, the Z^0 and the W^+ and W^- , the predicted quanta of the Salam-Weinberg unified theory of the weak and electromagnetic interactions. Testing this theory is now recognized to be crucial. Success will give confidence to more ambitious attempts to construct unified field theories. Failure, however, would also be suggestive.

CERN's proposal is strictly for "LEP phase 1", which involves building a tunnel so large that ultimately beams of 130 GeV could be retained (more than enough to create $W^+ - W^-$ pairs), while cutting back on accelerating cavities (which inject the energy into the beam). The result is that LEP phase 1 would reach 50 GeV per beam, enough to make the Z^0 and make the initial tests of the Salam-Weinberg theory.

LEP phase 1 also cuts back on experimental facilities: only four of the possible eight experimental halls would be equipped. And it proposes a new injection system, which makes use of existing accelerators at CERN and eliminates the need for a large electron synchrotron, which would have been the biggest in the world, merely to inject electrons into LEP. In contrast, the intersecting storage rings, one of CERN's greatest technical successes, will not now be needed for the electrons and will be devoted to experiments outside subnuclear physics.

Professor Schopper, who is at present director of the German electron physics laboratory DESY near Hamburg, said this week that it was most important that the CERN Council had recommended that LEP be built out of CERN's running budget rather than as a separate programme. A director has been appointed to act as LEP project leader, as soon as governments give approval; this is Dr Emilio Picasso, a prominent Italian physicist at CERN. The project leader, said

Schopper, would be able to organize and draw on talent throughout CERN, for short and long periods, avoiding the career problems and inflexibility that would come with a new "LEP division". "We will bring the work to the people", he said.

The CERN Council has adopted a "firm" budget for 1981 of 596 million Swiss francs (£156 million), but this includes no provision for the construction of LEP. Schopper will propose a running budget which will include LEP in six months' time: he expects it to be a little larger than the 1981 budget "but we are only talking of a per cent or two". CERN staff are now working on reducing the cost of individual LEP components. The level of the budget will determine not whether, but how fast, LEP can be built.

● CERN's 25-metre-square antiproton accumulator is to receive its first particles in the next few days — less than two years after the ground was first broken. The accumulator will be tested with protons, but the intention is to use it to reduce or "cool" the transverse motions of antiprotons created in proton-target collisions. The antiprotons, a few per collision, will be accumulated and then injected into the 400 GeV super proton synchrotron counter-current to the SPS proton beam.

Robert Walgate

Radioactive waste

Polite debate

A first attempt by the UK National Radiological Protection Board and the British Association for the Advancement of Science to stimulate public discussion on the problems of radioactive waste management was a damp squib. A meeting they organized on 18 May, attended by interested parties in the nuclear industry, government agencies and the anti-nuclear groups, did little to uncover the detailed technical problems.

Part of the reason for the low key in which the meeting was conducted appears to have been the decision of the British authorities that, even with the new nuclear programme, fresh arrangements for long-term disposal will not be needed until the end of the century.

Both intermediate and high-level waste have been stored at Windscale for 25 years and could sit there for many more without the authorities becoming unduly bothered. If anything, the intermediate waste is a more urgent problem — it accumulates fairly rapidly and storage facilities are costly. High-level waste disposal is nevertheless receiving most attention. Out of a total waste management research budget of £25 million, the UK expects to spend more than £10 million this year on one method of treating high-level waste: the HARVEST vitrification process which has been under development by the UK Atomic Energy Authority since the late 1950s. The British government plans a

prototype vitrification plant operating at Windscale by 1990 if the go-ahead for the plant can be given some time this year.

The decision for British Nuclear Fuels, which runs Windscale, will not be easy, however; it will have to choose between its own HARVEST process and the French equivalent (AVM) which has been operating commercially since 1978. Officials are keen to point out that a decision to opt for AVM would not mean the end of HARVEST, which should be kept going to keep later options open.

The government plans to start producing vitrified high-level waste by the early 1990s for several years' storage and then final disposal in some sort of demonstration facility at the end of the century. There are three options for final disposal — on or under the ocean bed or in deep geological structures.

The Radioactive Waste Advisory Committee, a group of independent advisers, recommended in its first annual report, published earlier this year, that research on the first two options should be increased.

The most promising option seems to be disposal in deep geological structures. The government programme of test-drilling in various rock formations throughout the country, however, has excited considerable public opposition. The UK Atomic Energy Authority, which started the programme now under the aegis of the Department of the Environment, has so far been able to drill boreholes at only one site in Scotland.

Planning permission for three others has been refused and the authority is awaiting the results of appeals. The Institute of Geological Sciences is now making preliminary surveys of about fifteen other sites for the Department of the Environment in the hope of drilling at some of them fairly soon.

Judy Redfearn

Russian language

Finns struggling

Recent cutbacks in university Russian departments, the falling off in the numbers of school-children sitting for Russian Advanced-level examinations in secondary schools and the virtually overnight decision to close down the Russian department of the University of Lancaster have caused considerable concern in British academic circles. Very different, one might think, the situation in Finland, where a recent report of the Ministry of Education urged substantial increases in the teaching of Russian.

According to a memorandum submitted last month to the Minister of Education, Paer Stenbaeck, at least one group of students is however at risk. The memorandum, drawn up jointly by the Finland-USSR Society and the Fund for the Promotion of the Study of the Russian language claims that a "worsening" of the position of Russian language teaching in Finnish secondary schools could be a

serious handicap to university students reading mathematics and science.

The report sets a target that 30 per cent of the active population (people between 20 and 63) should have a knowledge of Russian — thus giving Russian, for the first time, parity with German in importance as a means of international communication.

Language study in Finnish schools is complicated by the existence of two national languages — Finnish and Swedish — one of which has virtually no use and the other only limited use as a means of international communication.

According to the new proposals, all school-children must learn one foreign language (at present this is usually English) and the "other national language" (Swedish for Finnish-speakers and conversely). Study of a third language is optional, and introduced in the eighth class (15 plus).

According to the 1977 figures, some 0.2 per cent of school-children learn Russian as their first language and 6.2 per cent as their third language. The committee's recommendation of a 30 per cent Russian-speaking "active population" seems, therefore, an improvement. Their recommendations, however, refer to the whole range of language teaching — including, for example, business and secretarial courses. The "pro-Russian" lobby maintains that the school timetable is now so overburdened that few science-orientated students will be able to fit in a third optional language and will, accordingly, have no access to Russian scientific literature.

To the present generation of Finnish scientists, however, a lack of knowledge of Russian seems no particular burden. They use the cover-to-cover English-language translations of Soviet journals, and have little need, they say, for spoken Russian as a medium of scientific communication.

Vera Rich.

Experimental animals

Lab invaded

The UK Agricultural Research Council's Institute of Animal Physiology at Babraham, near Cambridge, has lived under threat of physical attack by anti-vivisectionists for several years. Last weekend the expected happened when a group of demonstrators forced their way into administrative buildings and laboratories housing animals. One object of the invasion was apparently to photograph animals in the laboratory.

The cost of the damage is small — no scientific experiments were tampered with. Preliminary estimates put it at a few hundred pounds. But, says the ARC statement, the noise of the break-in and the flashing bulbs of cameras must have caused alarm to the animals, which are accustomed to good treatment.

After some reports in the press earlier this week, the ARC also said that neither "outsize" goats nor crossbreeds between goats and horses are kept at Babraham. Some demonstrators questioned on the site were apparently under the misapprehension that toxicity tests with cosmetics and cigarettes were being carried out.

The Institute of Animal Physiology has been a focus for the anti-vivisectionists for some time. The institute has pointed out before that all experiments done there are subject to licensing by the Home Office and are not exceptional in that similar experiments take place in other laboratories.

Judy Redfearn

Whaling quotas

More wrangling

The 1980 general meeting of the International Whaling Commission at Brighton on 21-26 July will have on its agenda a call for a total moratorium on whaling. This is the third time that the issue has been raised; on previous occasions, however (1972 and 1979), the motion was amended to one for selective moratoria. This year, the proposal for a total moratorium is tabled, *inter alia*, by France, the Netherlands and the United States, and is felt to have an even chance of success.

As in previous years, defeat or amendment of the motion would probably result not from a change of heart by the pro-moratorium lobby but from a feeling that adoption of such a motion would push anti-moratorium countries out of the convention and therefore beyond international control.

The way in which the argument at Brighton is likely to go can be told from what opponents of a total moratorium are saying in advance. One of the strongest opponents of a total moratorium, the Soviet Union, recently made its position

clear in an English-language broadcast on Moscow radio. Calls for a total moratorium, said commentator Boris Belitskii, have no ecological basis, but are based on emotional attitudes encouraged in some quarters for political reasons.

The English-language broadcast added that in the case of really endangered species and in certain limited areas the Soviet Union has cut back its whaling considerably. (In the case of the baleen whales of the Sea of Okhotsk, a 2-year total ban has restored the population from the verge of extinction.) The broadcast went on to say that, over the past few years, the Soviet Union has closed down on-shore whaling factories and has disbanded the whaling fleet based on the Baltic port of Kaliningrad, dispersing the ships to marine and ichthyological research.

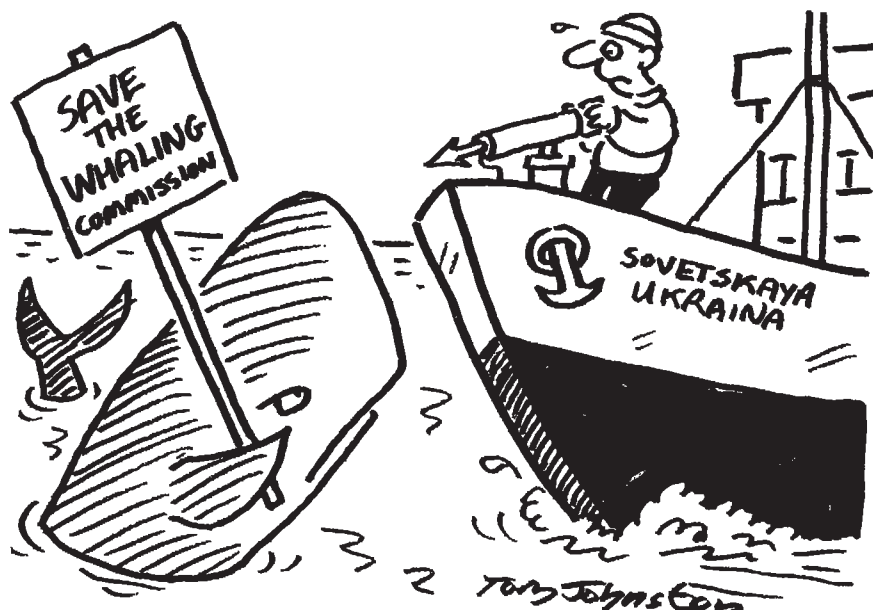
At present, Soviet whaling operation are said to be based on Vladivostok, and the two flotillas, with mother ships *Sovetskaya Rossiya* and *Sovetskaya Ukraina*, carry the necessary international observers as well as Soviet marine ecologists.

Rhetoric apart, the Soviet Union and other anti-moratorium countries such as Japan appear to have a certain case: they joined, they say, a "whaling" commission (to control and maintain the industry) not an "anti-whaling" commission.

There has been considerable speculation that, should the pro-moratorium advocates carry the day at Brighton, the Soviet Union and Japan might set up a rival commission of their own. Already the two countries have had some bilateral cooperation over quotas.

When, last year, the Soviet Union accepted the IWC's decision for a partial moratorium, in which fishing from factory ships would be confined to minke whales, it implemented this decision by ceding to Japan its North Pacific quota of 230 bryde whales in return for 300 of the less valuable minke whales from Japan's quota in the Antarctic.

Vera Rich



Big science

German doubts

WEST Germany is in the process of reviewing ten major scientific projects which would cost more than £500 million if all were funded now. They range from a continental deep drilling project which would reach the Mohorovic discontinuity at a cost of £30-75 million per hole to the possible commitment to the European proposal to build a large electron-positron ring (LEP) at CERN, Geneva at an estimated cost to Germany of £90 million.

The German research ministry, the BMFT, has set up an *ad hoc* committee of seven distinguished scientists and one journalist (to give "the people's view") which has already begun taking evidence. The cases have been heard for LEP, HERA (an electron-proton ring proposed for the DESY laboratory near Hamburg) and a relativistic heavy ion accelerator (10 GeV per nucleon) for the nuclear laboratory GSI at Darmstadt.

The chairman of the committee, Professor Klaus Pinkau of the Max-Planck-Institute für Physik und Astrophysik, Munich, said last week that he would make "a brave attempt" to finish all the hearings before August; the committee's views are not requested before mid-1981, but the sooner they are received by the new government after the October 1980 general elections, the more likely they are to affect policy, Pinkau believes.

It is not possible, says Pinkau, to make a linear list of priorities through the projects; rather the committee must "model a research landscape". The committee has been given no financial guidelines, so that it will be "a piece of artistry" to work out what the government can afford. Pinkau sees the committee as one lying between scientists and government rather than a part of government: it will make judge-

ments among the projects on their merits, and then turn around and plead with government on behalf of those it supports.

Despite the strength of the German economy and the scale of the package of proposals now being reviewed, financial pressures are now making themselves felt. Recently, the minister for universities, Dr J Schmude, said in a speech at the Deutsches Forschungs Gemeinschaft (which funds most German university research) that scientists would have to be prepared to spend less. The national budget is being pressed by the world recession, the increasing defence budget and an increased contribution to the European Community budget to offset Britain's soon-to-be-reduced subscription as negotiated by Mrs Margaret Thatcher. "Research won't be exempted" from the cuts to come, said Pinkau.

One result will probably be that the biggest projects on the list will not be funded simultaneously. And the most direct conflict now seems to be between HERA (for Hamburg) and the relativistic heavy ion accelerator (for Darmstadt). LEP is an international project which would be paid for if the current budget for CERN at Geneva can be maintained at something like its present level. Germany has said that, on terms like these, it will not object.

HERA (electron-proton) and GSI (relativistic nuclei) are, however, both national projects in closely adjacent fields. HERA would probe the structure of the proton and the quark, and the GSI device would create, for example, regions of extreme nuclear density which in principle could simulate neutron star conditions (although it would be difficult to extract the relevant information from the experiments). And, said Pinkau cryptically, "one should be aware that DESY", where HERA would go "is an internationally important laboratory". **Robert Walgate**

Genetic manipulation

Soviet claims

On at least two occasions in the past month, the foreign services of Moscow Radio have put forward the startling claim that a team of geneticists at the Institute of Genetics and Cytology of the Byelorussian Academy of Sciences in Minsk have succeeded in manipulating the symbiotic bacteria of cereals to yield nitrogen-fixing properties.

The claimed success is surprising, especially because Minsk has no particular renown as a centre for genetic engineering, and even the most prestigious of the Comecon molecular biological establishments — Szeged — admits that its own work in this field still has a very long way to go. There is, however, a possibility that the reported discovery was not made at Minsk at all.

Indeed, the claim seems to be based on a paper by a team headed by M.A. Troicki entitled "Nitrogen fixing activity of hybrids between *Bacillus oligonitrophilus* and *E. coli*" (*Viesci Akademii Navuk BSSR, Serija Bijalahichnych navuk*, 3, 1980). This describes a fairly routine procedure — nitrogen-fixing hybrids of *E. coli* with soil bacteria were produced at the University of Sussex nitrogen fixation unit as early as 1972, and the only novelty seems to be the use of *B. oligonitrophilus*, a name unknown to Western taxonomy, but which Troicki *et al.* state that they themselves isolated from the soil.

The authors themselves claim merely to have demonstrated the possibility of transferring nitrogen fixation genes from *B. oligonitrophilus* to *E. coli*, although the fact that their paper was published in Russian, whereas previous papers had appeared in Byelorussian, may suggest that they felt it merited All-Union rather than local attention.

Why, then, did TASS make such inflated claims? It is possible that the TASS science commentators simply misunderstood the paper, though it is difficult to see how they could do so, since Troicki's team does not mention cereal crops at all — the work reported relates to nitrogen fixation *in vitro*. It is not impossible, however, that the claim, coming at this particular time, has political overtones.

The first announcement was made on 25 May. The following day, *Pravda* published a major article on the Warsaw conference of the Warsaw Pact states entitled "Disarmament: Real Measures are Possible". Ostensibly an appeal for military detente, it contained claims that the United Kingdom and the United States are stepping up work on bacteriological weapons. In particular, said *Pravda*, the United States is working on "ethnic viruses" — bacteriological weapons which would selectively attack members of particular races or ethnic groups.

The case is cited of a black American, Eli

Major projects under review

	Cost (million DM)*	Timescale (yr)	Proposer
Spallation neutron source	400-570	8-15	Karlsruhe and Jülich
BER II reactor improvement	47	2	Hahn-Meitner Inst., Berlin
Relativistic heavy ion accelerator	190	6	Darmstadt
Superconducting nuclear cyclotron, 250-300 MeV per nucleon	a) 44 b) 33	4-6	a) Jülich b) Technical Univ., Munich
Ion beam fusion accelerator	100	5	Karlsruhe; <i>proposal withdrawn</i>
LEP	350 (for Germany)	6-8	CERN, Geneva
HERA	600	7	DESY, Hamburg
European synchrotron radiation source	100-230	5	European Science Foundation
Research vessel	75	2	DFG; to replace 'Meteor'
Continental deep drilling	120-300	1-5	DFG; price is per hole

* £1 sterling = 4.14 DM

Mayji, who allegedly developed a throat tumour after working as a guard at a secret US naval installation at West Oakland, where, it was alleged, Rift Valley Fever, which causes inflammation and malignant tumours, was used as a basis for an "ethnic" weapon.

Such allegations are not entirely new. Reports of alleged research on an "ethnic bomb" and other bacteriological weapons were put out by Soviet radio in 1978 (apparently in connection with the Robert Toth affair) and again earlier this year, to counter Western intelligence reports of a fatal epidemic in April 1979 emanating from a Soviet military bacteriological research station outside Sverdlovsk. (Soviet official sources said the incident was a natural outbreak of anthrax.)

Hitherto, the specific bugbear of a US "ethnic" killer has been restricted to transmissions on "Radio Peace and Progress", the Soviet service beamed to the Third World. The appearance of such charges in *Pravda*, the leading Party newspaper, is a new departure.

Vera Rich

Dioxin

ASTMS says no

The UK Health and Safety Executive came in for some sharp criticism last week from the Association of Scientific, Technical and Managerial Staffs (ASTMS). The union, with a strong following among scientists, accused the HSE of being "sluggish and ineffective" in its handling of the case of workers exposed to dioxin during an explosion at the Derbyshire plant of the Coalite and Chemical Products Company in 1968 (*Nature*, 6 March).

In 1976, after a similar accident at Seveso, Italy, the Employment Medical Advisory Service of the HSE persuaded the Coalite company to carry out studies of the workers exposed in the accident. Full reports of those studies, however, were not released to the HSE until last April in spite of several previous requests from the medical advisory service for all the information available.

In its document "Report on Coalite Chemicals", published last week, ASTMS criticizes the advisory service for not pressing harder for the earlier release of the studies. Results published in *The Lancet* early last year, it says, were sufficiently at variance with a summary of the studies released by the company to have persuaded the HSE to invoke its powers to secure the information. The HSE, however, says that increasing its pressure on the company would almost certainly have involved a lengthy legal case. Since manufacture of 2,4,5-T, of which dioxin is a contaminant, had already stopped at Coalite — indeed everywhere in the UK — the HSE did not consider release of the reports as its first priority, preferring instead to concentrate on current hazards.

The ASTMS document is also critical of

the way in which Coalite conducted the studies. There was no properly matched control and not all the exposed workers were included, which means, says ASTMS, that little weight can be attached to the interpretation of the results.

The HSE is a little peeved that ASTMS appears to put the blame for these inadequacies on its shoulders, holding that Coalite must be held responsible. Both ASTMS and the HSE are agreed, however, that further studies need to be done. ASTMS is suggesting that they be carried out independently of Coalite and the advisory service under a joint union-steering committee. The advisory service is however persuading Coalite to re-do the studies, this time under tighter control.

Another specific point which ASTMS takes up is the lack of union participation at two key meetings. One was held last November to discuss the occurrence of chloracne, the disfiguring skin disease caused by dioxin and some other related chemicals, at three companies — Coalite, Stavel Chemicals and Monsanto in South Wales. According to ASTMS, the TUC medical adviser had pressed for union involvement in this meeting throughout 1979, but after some delay the HSE group finally decided that the meeting should be for "doctors only". The second meeting was held on 1 May between the advisory service and the Coalite workforce and management to discuss the preliminary assessment of the studies. ASTMS complains that the first it knew of the meeting was from that day's *Nature*.

All in all, ASTMS now says it has lost confidence in the HSE to make sure that sensible studies on workers are carried out. It asks that the HSE should compile a full report for the unions of the extent of recent contact with chloracnogenic chemicals, that the TUC and the HSE should draw up guidelines for union involvement in future studies of workers and that they should discuss what to do now about Coalite.

In the midst of these specific criticisms, the ASTMS document says that it is important not to lose sight of the most important issue, that of the inherent dangers of dioxin and the herbicide 2,4,5-T. It has now joined the growing group of unions calling for a complete ban on import into and use of the chemical in the UK. The most important thing, says Clive Jenkins, president of ASTMS, is to "get rid of the stuff". **Judy Redfearn**

Arctic voyage

Swedes set out

Stockholm

The Swedish icebreaker "Ymer" sailed for the Arctic on 24 June on an expedition which has been delayed for more than a year because of Russian sensitivities about the strategic importance of the Arctic Ocean. When the ship left last week, it carried a team of biologists; it will return

later in the summer to pick up a group of earth scientists.

The expedition was originally planned to celebrate the centenary of the first circumnavigation in 1897 of Europe and Asia by the Swedish explorer Adolf Erik Erik Nordenskiöld, who began his voyage by sailing from the Atlantic to the Pacific along the Siberian coast, through the Northwest Passage. The original plan had been to retrace this route as far as the Bering Strait, and then to circumnavigate the Arctic Ocean.

Permission was asked of the Russian authorities to sail along the Siberian coast and to take sediment cores from the continental shelf there. Although the Swedish Prime Minister Fälldin took up the matter with Mr Kosygin, the Russians never replied. Reconciling themselves to Russian views about the strategic importance of the area, the Swedes changed their route.

Under the revised plan, the "Ymer" will sail through the northern Barents Sea, the deep ocean north and northeast of Spitsbergen, the sea between Spitsbergen and Greenland and the waters off northern Greenland, some never sailed before. The research is being financed by the Natural Sciences Research Council, and will involve about 80 scientists from eight countries.

One of the chief objectives will be to tell to what extent the Arctic is polluted. An increase of the lead content of Greenland snow and ferns has been recorded after the introduction of high octane petrol in the USA; but the origins and extent of other pollutants (aerosols, gases and particles) in the Arctic atmosphere, are not known. Atmospheric pollution may however affect the delicate radiation balance of the Arctic, which could in turn affect climate in neighbouring latitudes.

The expedition also plans to investigate the cooling during the Arctic summer of the lower atmosphere under the influence of the ice-pack. The formation of stratus clouds is thought to influence radiation balance and thus the climate.

The Arctic climate is also affected by the movement of huge bodies of water. Large amounts of warm, salty Atlantic water flow north along the west coast of Spitsbergen to the Arctic Ocean, while a mass of cold and less saline Arctic water flows south along the east coast of Greenland. Oceanographers with the expedition will collect information on temperature, salinity and currents down to great depths. They will be able to trace water movements in the Arctic basin by measuring concentrations of radioactive pollutants from the reprocessing plant at Windscale in the UK.

The expedition hopes to be able to determine the extent of the Quaternary ice sheet in the European Arctic. From earlier expeditions to the area, glaciologists think that an earlier Arctic ice sheet reached the Barents Sea, but they have not yet been able to tell whether it was ever joined to the Scandinavian ice sheet. **Wendy Barnaby**

CORRESPONDENCE

Mr Arthur Frank

SIR, — The implications in Judy Redfearn's article (29 May) must not be allowed to pass unchallenged. I cannot speak for other directors but I trust that you will correct some of the misconceptions in the piece insofar as they may be thought to apply to Mr Frank's relations with the National Army Museum.

Three years ago he presented 80 optical and other instruments used by the British Army. They came free of any charges or conditions. This year the museum again benefited from Mr Frank's generosity when we received another 33 instruments. The second series came with the painless condition that they are on loan until such time as they can be put on display. They will then become the inalienable property of the museum, an invaluable addition to a section of the museum's collections which was extremely weak.

The caption "Arthur Frank with his instruments: gift horse or salesman?" is as unjust as it is clumsy. It is misleading, offensive and unworthy of a journal of the standing rightly enjoyed by *Nature*.

Yours faithfully

WILLIAM REID

National Army Museum,
London SW3, UK

Book prices again

SIR, — Dr Brindley (12 June) points out that paperbacks are published, on the average, at less than half the selling price of the cloth edition of the same title. He suggests that publishers of scientific books should make paperbacks more widely available to increase sales and perhaps profits.

The actual difference in cost between producing a paperback and cloth edition is about 65p for a book of 300 pages. What most publishers do is to subsidise the paperback edition by overpricing the cloth edition; this also happens in the pricing of journals where libraries have to pay an inflated rate which subsidises the cost of supplying copies to members. It should be understood, therefore, that where there is a dual edition the paperback is made available to individuals at an artificially low price at the expense of libraries. There is a strong feeling among publishers that this is just as most libraries are still allowing widespread photocopying which must reduce sales.

Yours faithfully,

ROBERT CAMPBELL

Blackwell Scientific Publications Ltd.,
Oxford, UK

SIR, — The comparison of typical paperback and hardback book prices tabulated by G W Brindley (12 June) is illuminating, but I could hardly disagree more strongly with the conclusion which he draws from these data. The fact that paperback editions are usually much cheaper does not necessarily imply that it would be in the public interest for them to be made yet more widely available. It would surely be infinitely preferable to persuade publishers to adopt a pricing policy which honestly reflected the difference in unit costs (ca £1-£3) of the two types of binding.

An alternative way of looking at G W Brindley's figures is to work out, in each case, the publisher's pretended costs incurred in providing hard covers as opposed to paper: the booby prize clearly falls to Elsevier, who seek to extort from the unfortunate purchaser an astounding \$45.75, merely for putting covers

on their *Practical methods in electron microscopy*, followed by U California Press who price the covers of *Mezozoic mammals* at an almost equally surprising £15.25. It is admittedly unfair to make this sort of comparison without taking proper account of the comparative length, page size, quality and so on of the books in question; but this hardly alters the central conclusion, which is that the alleged cost of hard covers bears remarkably little relation to reality.

The explanation, one is told, lies in the publishers' naive belief that libraries will always purchase hardback editions, virtually regardless of cost, so that profits can supposedly be maximised by raising the hardback prices to increasingly absurd levels. The ordinary reader is then offered an edition identical in every respect except that it is bound between paper covers and carries a rather more realistic price tag. The fact that he or she would often prefer to pay an extra pound or so in order to gain the durability and convenience provided by a hard cover, is conveniently ignored.

There appears to be little justification for this abominable practice, which is a continuing source of despair for bibliophiles and of frustration for practising scientists. It is surely high time that it was brought to an end. Is there really no hope of persuading publishers to move back towards a pricing policy showing some degree of sensitivity towards the interests of ordinary book buyers?

Yours faithfully

P V E McCLINTOCK

Physics Department
University of Lancaster, UK

Parkinson extended

SIR, — In his letter "Expanding on Parkinson's law" R. Moss produces the relations $I_{ai} = W^2/A + A$ where I_{ai} is the workload of a single administrator, W is the number of productive workers and A the number of administrators, for the most advanced state of any organisation. He then suggests that this relationship will lead to a limiting ratio of one administrator for each productive worker. Under some conditions, however, the relationship gives rise to a bureaucracy which expands without limit.

The workload of a single administrator, I_{ai} , falls to $2W$ as $A \rightarrow W$ and increases thereafter. If the objective of the chief administrator is to minimise I_{ai} , the number of administrators will tend to W , but this will not be the case if instead the chief administrator seeks to increase the number of administrators until some aspiration level O is reached. If O is greater than $2W$, recruitment will slow down before the Moss ratio is reached. If, on the other hand, O is less than $2W$, the individual workload of an administrator will never reach the aspiration level, no matter how many administrators are employed, and recruitment will proceed at an ever more furious pace as more administrators are needed to meet the demands of an ever-increasing workload. Even if the chief administrator does attempt to minimise individual workloads, should the number of administrators ever exceed the number of productive workers the chief administrator will find himself appointing still more administrators in an attempt to regain his previous workloads.

The Moss ratio can thus be envisaged not as a limit on the number of administrators but as an event horizon beyond which a bureaucracy is doomed to expand ever more rapidly in an attempt to cope with the ever-increasing flow of minutes and memoranda generated within its own bounds. Ultimately it is unable to cope even with that function and becomes a

veritable black hole of forms and minutes.

Yours faithfully

J. C. THORPE

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Manchester Polytechnic,
Manchester, UK.

Ethnographic DNA

SIR — Several of the articles in your issue of 26 June note that both confusion and entertainment can be had by considering DNA as selfish, ignorant or, indeed, both. Perhaps these terms are not so much anthromorphic as ethological. Competing DNA molecules are, after all, made up of four main nucleotides, the deoxyribonucleotides of that famous intratribal totem sub-group A, T, C and G. We know from previous analysis of intra-tribal totem preferences that totem clan A is matched by totem clan T, and so is totem clan G with C. Commonly called base-pairing, it is of course fully understood that neither a moral imputation is implied by base, nor sexual by pairing.

We have yet to learn what resources competing DNA molecules had thermodynamically to write over, but it may very well be that the totems of A, T, C and G hold secrets. For example, codons with their middle base T preferentially code amino acids with polar side chains, while those with A as centre base in the triplet preferentially code non-polar side-chain amino acids. There appears little bias towards polar or non-polar by codons with C or G as the triplet centre¹. Closer inspection reveals that A is replete with two amino groups and T with two hydroxy groups on the base nucleus; G has an amino and a hydroxy, so does C. As Kipling noted, there are 99 ways of making tribal lays — but in this tribe why should a totem of -NH₂ mean non polar coding, and -OH polar? What happens to totem loyalties when -NH₂ and -OH are paired, in C and G? The strong totem preference is annulled — are we seeing cultural impoverishment by mere group composition?

Since it appears that we have no answers to these questions at the anthropomorphic, ethological or any other level, it may be salutary to recall that molecular biology is about molecules — not selfish this or that. If this trend continues we can expect to see defrocked DNA and rampant tRNA.

Yours faithfully

ANTHONY HARRIS

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London W3

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Conversion courses

SIR, — My attention has been drawn to the reference in Robert Walgate's article on Dr Shallis (7 February) to our conversion course from arts to sciences and to the fact that it has closed. Sadly this is true; it closed seven years ago for lack of applicants. Not all our students were as successful as Mike Shallis, but a number are now active as scientists. We demonstrated that with motivated students it was possible and not too difficult to effect such a conversion, and we gained experience in the ways of doing it and the pitfalls to avoid. Although the course has closed, the experience is still there and available to anyone who wishes to start such a course again. There is surely need for it as long as our schools force children into choices at an age too early for them to know their minds.

Yours faithfully

L R B ELTON

University of Surrey,
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NEWS AND VIEWS

Cell cycle control — both deterministic and probabilistic?

from Paul Nurse

ONE of the basic questions of cell biology is what determines the rate at which cells undergo division. Currently there are two models of cell cycle control which provide answers to this question. The first is the deterministic growth controlled model which developed mainly from experiments with microbial cells, and the second is the transition probability model which developed to explain the behaviour of mammalian cells.

The basic postulate of the growth controlled model is that the rate of division is determined by the rate of growth of the cell¹. Some cell cycle event cannot take place until the cell has attained a critical cell size, and as a consequence the rate at which cells grow to that size determines the rate at which they divide. The model very simply explains why in steady state populations of exponentially growing cells, size at division remains constant and the rate of division parallels the rate of increase in cell mass. It is further supported by observations that the occurrence of certain cell cycle events, such as the initiation of DNA replication in *Escherichia coli*², traverse of 'start' as marked by bud emergence in budding yeast³, and cell division in fission yeast⁴, are well correlated with the attainment of a particular cell size.

In the original form of the Smith and Martin transition probability model, the cell cycle is composed of an A-state and B-phase⁵. In the A-state the cell does not progress towards division but it has a constant transition probability of entering the B-phase. The B-phase is made up of most of the conventional cell cycle and is completed in a constant period of time. Therefore the rate of division is determined by the time a cell takes to undergo the random or probabilistic transition from the A-state and to the complete the constant time period in the B-phase. The main evidence in favour of the model is that the distribution of cell cycle times includes an exponential component which reflects the probabilistic transition from the A-state. This evidence is particularly

strong if it is assumed that the B-phase is identical for sister cells, since the differences in cell cycle times between sister cells can be described solely in terms of an exponential⁶. The deterministic or growth controlled model cannot easily explain such a distribution⁷, although with further assumptions it can do so (for example see reference 8).

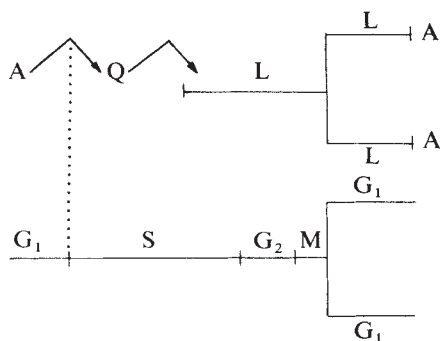
Brooks *et al.*⁹ have recently modified the transition probability model to account for the identical B-phase in sister cells, and to deal with the behaviour of cells stimulated from quiescence. In the modified model, cells begin their progress towards division by a random transition from a Q-state to commence a process L. This process takes a constant time period to be completed after which the cell enters the A-state. Exit from the A-state is at random and leads to DNA replication and subsequently cell division, and, in addition, to entry into the next Q-state. In the modified model the time taken to complete the two random transitions and constant time period of the sequence Q→L→A determines the rate of cell division. The model explains why the differences in cell cycle times between sister cells can be described solely in terms of an exponential since the Q→L part of the sequence occurs in the mother cell and thus is common to the two sister cells. Consequently the differences between sister cell cycle times are due solely to the random A-transition which results in the exponential distribution. The B-phase does not feature in the modified model, but its duration is equivalent to the Q→L sequence accounting for the identical B-phases of sister cells. The modified model also explains why there were two points of control in stimulated quiescent 3T3 cells¹⁰, since one control was acting at the Q-state transition and the other at the A-state transition. Brooks *et al.*⁹ have also suggested that the behaviour of the mitotic

centres organising the spindle poles could act as an analogue of the Q→L→A sequence⁹. The initiation of duplication of the centres could correspond to the Q-state transition, their maturation to the L-process, and their separation to the A-state transition. This suggestion is interesting, since centrioles are likely to provide a convenient marker for the behaviour of the less tangible mitotic centres⁹, and centrioles or their equivalents have been implicated in cell cycle control of both 3T3 cells¹¹ and budding yeast cells¹².

The modified transition probability model is an advance on the original form of the model but suffers from one or two difficulties. In rat hepatoma cells van Wijk *et al.*¹³ have found that most of the variability in cell cycle times between sister cells is due to variability in the duration of G₂. This is difficult to reconcile with the A-state transition occurring before S-phase. In addition, the duration of the B-phase (equivalent to the Q→L sequence) is more similar for cells of rat hepatoma which share a common grandmother (cousins), than for unrelated cells¹⁴. The modified transition probability model predicts that sister cells should have identical B-phases, but that cousin cells should be as dissimilar as any pair of unrelated cells within the population. Finally, centriolar behaviour may not be such a good analogue of the Q→L→A sequence, since the model predicts that quiescent cells in the Q-state should only have a single centriole, whereas Tucker and Pardee¹¹ have shown that 3T3 cells in such a state have a pair of centrioles.

The deterministic growth controlled model and the transition probability model give apparently different answers to the question of what determines the rate at which cells divide. One explanation for this may be that the cell cycle controls are quite different for microbial and mammalian cells. However, some experiments suggest that this may not be the case. The rate of bud emergence has been followed in cells of

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After an indeterminate period of time in the A state, a cell makes the transition to the Q state and at the same time starts synthesizing DNA (S phase). The cell remains in Q for an indeterminate period before making the transition to L. If the transition occurs during S (as shown here) or G_2 , the L state, which has a fixed time span, will last until after mitosis (M) and division. Therefore both sister cells will start their own A state simultaneously.

budding yeast that have been released from a block at 'start'¹³. The distribution of times to bud-emergence within the population includes an exponential component as would be expected from the transition probability model. To explain this observation and the cell size correlation with bud-emergence mentioned above, Shilo *et al.*¹⁵ suggested that traverse of 'start' requires growth of the cell to a critical size followed by a random transition which 'started' the cell cycle. A rather similar conclusion has been arrived at by Shields *et al.*¹⁶ from investigations of the role of cell size on cell cycle time in mouse fibroblasts. Quiescent small cells take longer to enter S-phase than do large ones, and cells born of small mother cells have longer cell cycle times than those derived from larger mothers. The distributions of the times required before S-phase and cell division took place were found to include exponential components. The authors proposed that cell size affects the length of the B-phase acting before G_1 , and within the framework of the modified transition probability model, that cell size affects the length of the L-process⁷. These experiments are suggestive that there is a size related element and a probabilistic element in the cell cycle control of both microbial and mammalian cells. Therefore it is possible that the deterministic and

probabilistic models are describing different aspects of a single cell cycle control. In microbial cells the probabilistic transition occurs very rapidly, and the deterministic size element is more important in controlling the rate of division. In mammalian cells the probabilistic transition is much slower, and the deterministic size element is of less importance in controlling the rate of division.

In this context it is of interest that some mechanisms for monitoring cell size and controlling the cell cycle include both deterministic and probabilistic elements¹⁷. For example, in one mechanism a structure is built up at a rate proportional to the size of the cell. Smaller cells will build up the structure slower than larger ones and consequently cells will tend to complete the structure at the same cell size. This is the deterministic size related element of the mechanism. Once completed, the structure then has to interact with some site within the cell to become active. Since there is only a single copy of the structure its interaction

with the site will be a random process and forms the probabilistic element in the control. If the building of the structure takes a long time then the control will appear deterministic and size related as in microbial cells, and if the interaction between the structure and site takes a long time then the control will appear probabilistic as in mammalian cells.

The possibility that the deterministic and probabilistic models describe different aspects of a single cell cycle control common to both microbial and mammalian cells, and that both aspects are important for determining the rate of division is at present based on few experiments. What is required are more kinetic investigations of the distributions of cell cycle times coupled with measurements of cell size. It would also be of interest to follow the behaviour of centrioles or their equivalent in the same experiments, to see if a firmer link can be established between these structures and the more abstract elements of the cell cycle control models. □

Can comets become asteroids?

from David W. Hughes

COMETARY nuclei, kilometric sized dirty snowballs, obviously evolve as the comets age. At each perihelion passage the nucleus will lose about a metre or so of dust and ice, producing the coma and the tail and also a large collection of meteoroids which feed the meteor stream. This process can continue until the nucleus has completely disintegrated into gas and dust but there are other possibilities. The nucleus could disrupt into a number of boulders which quickly outgas. The core of the nucleus could be slightly more compact than the outer regions and could remain when all the ice has sublimated. Or an insulating crust could develop which reduces the heat flow in towards the centre of the nucleus and thus stops the sublimation and the loss of volatiles. The last two possibilities lead to an apparent transformation of the comet into an asteroid. The Solar System would then contain active comets, asteroids and a third class of objects, extinct cometary nuclei which looks like asteroids but have the orbits of comets.

The problems of this evolutionary transformation have been discussed recently in two papers published in *The Moon and the Planets*. These are by Ľubor Kresák of the Astronomical Institute of the Slovak Academy of Science, Bratislava (22, 83; 1980) and H. Rickman, Astronomical Observatory, Uppsala, Sweden and C. Froeschlé, The Nice Observatory, France (22, 125; 1980).

Kresak stresses the role of Jupiter. The mean of the asteroidal orbits has the same

eccentricity and orientation as the orbit of Jupiter. Also there is a spherical region around Jupiter of at least 1 AU in radius which is completely void of asteroids. The system of short period comets on the other hand is aligned with the line of apsides of the jovian orbit and the immediate environment of Jupiter is filled with an overabundance of comets some of which even become jovian satellites for a time. Added to this fundamental difference in orbital characteristics is the problem of observational selection. In the case of comets we are dealing with a class of objects which have a potential lifetime very much shorter than the age of the Solar System. So the comets that are observed today are either the last, abnormally long lived survivors of a much more abundant original population or they must be a late generation of an evolutionary sequence.

The first of these hypotheses is unlikely. The second implies that we are observing an evolved state of equilibrium between source and sink and consequentially short lived objects are underrepresented.

It would also be very presumptuous of us to believe that all the types of asteroids and comets have been discovered. For example, asteroids were known for over a century before the Trojans (which follow Jupiter's orbit and have a mean elongation of 60° ahead and behind Jupiter) and the Apollo's (perihelion distance less than 1 AU) were

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discovered. In the last few years certain new classes of objects have been found, one with inclinations over 60° , another with periods less than a year. Chiron (asteroid number 2060) has an orbit between the orbits of Saturn and Uranus and considering the immense effect of distance dependent observational selection it is difficult to tell how many others there are in Chiron's class. One asteroid 1978 SB has been found to have an orbit similar to that of Comet Encke.

Observational bias is even stronger for comets because the brightness decreases drastically with heliocentric distance. Comets can only be seen if they get close enough to the Sun to display outgassing phenomena and to do this they have to get to within 0.0001 of the radius of the distant cometary reservoir known as the Oort cloud. Even considering temporal variations it is interesting to note that dependable astronomical observations have only been going on for about one ten-thousandth the average revolution period of a 'new' comet. At best we can only have seen 0.00001% of the whole comet population. A great number of long period comets must exist with perihelia beyond the orbit of Jupiter. Only five have been discovered so far, the first in 1974.

Even bearing these problems in mind Kresák concludes that very few of the asteroids could have been comets and none have been definitely identified as such. There are only two possible paths between the two orbital classes. Both require a complicated sequence of planetary capture by Jupiter or Saturn followed by strong non-gravitational forces to vary the aphelion distance thus pulling the comet away from Jupiter or Saturn into a more stable orbit undisturbed by planetary encounters. On attaining this stable orbit the non-gravitational forces have to cease.

The possible (even if non probable) ex-comets among the asteroids are Chiron, Hidalgo, Thule, members of the Trojan, Hilda and Grigua groups and the Apollo and Amor objects (the Amor's have perihelia between the orbits of Earth and Mars).

Rickman and Froeschlé specifically discuss the possible cometary contribution to the Apollo-Amor population. In 1963 Opik suggested (*Adv. Astron. Astrophys.*, 2, 219) that the perturbations of Mars on the inner boundary of the asteroid belt were too weak to account for the observed numbers of Apollo and Amor asteroids. A cometary origin was thus proposed for most of them. Rickman and Froeschlé

consider the steady state where all the Jupiter family of comets (aphelion distances between 4 and 8 AU) is a possible source, this family being fed by capture and depleted by deactivation. The deactivated comets are also in a steady state, their loss mechanism being expulsion from the inner solar system by Jovian perturbation. The number of Mars crossing deactivated comets is estimated by means of a Monte Carlo simulation of the orbital evolution of the Jupiter family under the influence of Jovian perturbations.

The authors conclude that no more than five percent of the short period comets can develop orbitally into Apollo-Amor asteroids after loosing their activity. Also no more than five percent of the nuclei of comets can contain sizeable asteroidal cores.

So even bearing in mind the problems of observational selection it seems that comets are comets and asteroids are asteroids. Even though it is possible in rare cases to perturb a comet's orbit into an orbit similar to that of an asteroid, there are no obvious dead comets amongst the asteroid population. This seems to favour the hypothesis that comets do not have asteroidal cores and decay completely into meteor streams. $\square$

The strong coupling constant is measured

from Mary K. Gaillard

THE initial evidence for hard gluon radiation by quarks came from the high energy electron-positron annihilation data of PETRA (*Nature* 281, 104; 1979). The continued accumulation of data has permitted quantitative measurements of this process, thus providing a direct determination of the strong coupling constant. In analogy with electric charge (which determines the interaction between electrons and photons), this constant determines the interaction between quarks and gluons.

According to the widely accepted quantum chromodynamic (QCD) theory of the strong forces, the dominant mechanism for hadron production is via quark-antiquark pair creation (Fig. 1a):

$$e^+e^- \rightarrow \text{virtual photon } (\gamma) \rightarrow q\bar{q}$$

However since quarks carry color charge they can radiate gluons, i.e. quanta of the color field. This is completely analogous to the well established quantum electrodynamic (QED) theory of electromagnetic forces where, e.g., the primary mechanism for μ -pair production

$$e^+e^- \rightarrow \text{virtual photon} \rightarrow \mu^+\mu^-$$

is dressed by photon radiation. The difference is that while photons and leptons can be detected in the laboratory,

quarks and gluons materialize as clusters ('jets') of hadrons, and sophisticated analyses must be performed in order to uncover the elementary QCD processes underlying what is actually observed.

The dominant component of gluon (or photon) radiation is soft or collinear with the quarks and its effects are submerged by the (so far) uncalculable angular spread inherent to the confinement mechanism governing the conversion of a quark or gluon into a jet of hadrons. Therefore most events should appear as two back-to-back jets (Fig. 1b), and this is indeed what was inferred from analyses of event configurations at SLAC several years ago, and directly observed more recently at PETRA where the higher energies produced jets sufficiently collimated to be recognized as such at a glance. Again there is an analogy with QED, where the dominant, very soft or collinear, component of photon radiation escapes detection through an inherent limit on measurement precision — except that for QCD the limiting precision is furnished by the dynamical effects of confinement.

However, sometimes in QED hard, large angle photons are emitted, but with emission rates suppressed by a factor of the fine structure constant $\alpha = e^2/4\pi$ for each

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such photon. In the same way, hard large angle gluons should be radiated by quarks with a suppression factor per gluon given by the strong coupling constant $\alpha_s = g_s^2/4\pi$, which is believed to be smaller than one. Therefore one expects some fraction of events to appear as three distinct jets of hadrons, a smaller fraction as four jets, and so on. Only at very high energies where jets are highly collimated can one hope to separate visually the clusters of hadrons in a multi-jet event, and the first three jet event, presented by the TASSO collaboration at the Bergen neutrino conference in June 1979, was obtained at a center-of-mass energy of about 30 GeV. A statistically significant sample of three jet events was presented by the TASSO group at the EPS conference in Geneva. By the Fermilab conference in August, the PLUTO and JADE groups had collected samples of three jet events where neutral as well as charged hadrons were measured, and the MARK J group also presented evidence for gluon radiation.

The relative production rate for three jet events provides a direct measure of the strong coupling constant α_s . Previous determinations of α_s were based primarily on the analysis of highly inelastic lepton-nucleon interactions where the dependence of observed phenomena on the strong coupling constant is considerably more

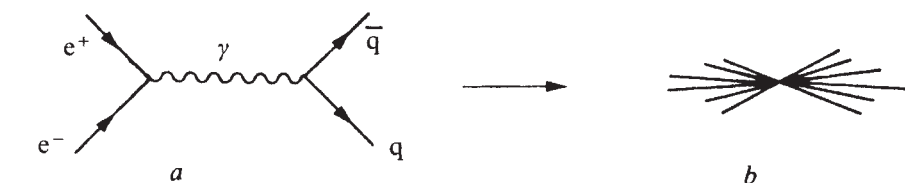


Figure 1

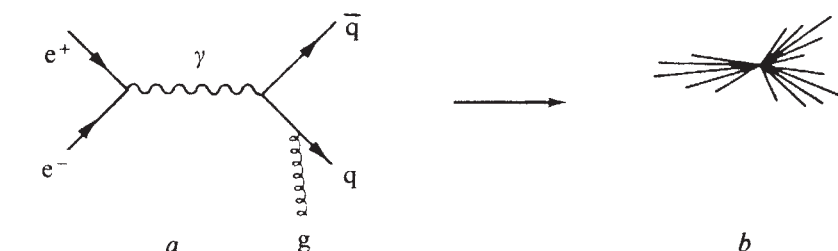


Figure 2

subtle. The method has been somewhat controversial as it requires measurements over a large energy range, including lower energies where the interpretation of the theory is somewhat problematic. On the other hand, a measurement of the rate for radiation of hard wide angle gluons at high energies is in principle straightforward and its interpretation is intuitively simple. However, a meaningful measurement of α_s requires substantial statistics, and since gluon (like photon) radiation prefers collinearity, even large angle radiation will most often produce events intermediate between Figs 1b and 2b, with two jets merged to form a single broadened jet.

The first task of the experimenters was to convince themselves that the collection of phenomena characterizing deviations from the two jet process of Fig. 1 could indeed be attributed to hard gluon radiation. There are various tests of this hypothesis, among them: a) the rate of jet broadening with increasing center-of-mass energy; b) coplanarity (up to the limited jet spread

due to confinement effects) of the final state hadrons which are supposed to arise from a primitive 3-quanta final state; c) asymmetric jet broadening — since the dominant deviation from two jet events is a single hard gluon emission, only one quark jet, the one with an accompanying gluon, should appear broadened. All four groups from PETRA presented analyses supporting these features at the Fermilab conference in August.

Since then more data has been taken, providing sufficient statistics for the first quantitative analyses published by the MARK J and JADE groups. The analysis of the MARK J group, based on the angular pattern of energy flow, rather than on an event by event reconstruction of jets, showed a statistically significant excess of oblate planar events which, when projected on the event plane and oriented so as to align the axes of maximum energy flow, displayed a three lobed structure which reproduced the 'antenna pattern' expected from single hard gluon radiation.

From the size of the effect they reported the first evaluation of α_s based on gluon radiation. This was soon followed by a value quoted by the JADE group based on detailed analysis of event shapes. More recently the TASSO group has extracted a value for α_s from a careful study of event topologies including various checks that their result is insensitive to the uncalculable confinement effects. The result of these analyses are consistent within their estimated errors of 20% or so and yield a value for α_s of about 1/5.

What does this number mean? Even without the complications of confinement effects which arise in QCD, one never really measures a fixed coupling constant, but rather the effective strength of some coupling among field quanta of specified energy and momenta. One then extracts a momentum dependent effective coupling; the momentum dependence reflects the effects of a higher order 'dressing' of the coupling through the exchange of virtual quanta. In QED these effects are tiny because the coupling is very weak, but in QCD they are appreciable, albeit calculable. A value $\alpha_s \approx 0.2$ for processes characterized by the PETRA energies where gluon radiation has been measured is quite consistent with expectations based on analyses of lepton-nucleon interactions mentioned earlier. More theoretical work, specifically addressed to higher order effects in electron-positron annihilation into hadrons, as well as more experimental work, allowing a determination of the momentum dependence of hard gluon radiation, are still needed for precision tests of the theory. Further studies are also needed to pin down the spin of the gluon and to measure the self-coupling of three gluons, a predicted feature of QCD which has no analogue in QED. □

Extra-pituitary actions of LHRH and its agonists

from Richard M. Sharpe

SINCE the origination of the concept of hypothalamic control of the anterior pituitary gland, it has been an accepted dogma of endocrinology that the releasing factors from the hypothalamus act solely on the pituitary to regulate the release of the appropriate trophic hormone. The vast number of animal experiments and clinical studies with LHRH since its structural characterization 9 years ago, have consolidated this belief. Now, a spate of recent results appears to have demolished this idea by demonstrating extra-pituitary inhibitory effects of LHRH or its agonistic analogues. The latter are considerably more potent *in vivo* than native LHRH, principally because of their resistance to enzymatic breakdown (Clayton *et al.* *Endocrinology* **104**, 1484; 1979), and this has been an important factor in permitting the demonstration of peripheral sites of LHRH action.

There have been numerous reports in the last 3 years demonstrating inhibitory effects of LHRH and its agonists on gonadal growth and function in intact animals, and LHRH agonists have exciting potential as new contraceptive methods in women, as luteolytic agents (Casper & Yen *Science* **205**, 408; 1979; Lemay *et al.* *Fertil. Steril.* **32**, 646; 1979) or, more probably, as inhibitors of ovulation (Bergquist *et al.*, *Lancet*, **ii**, 215; 1979). As it was already established that similar 'down-regulatory' effects could be achieved by overstimulation of the gonads with exogenous gonadotrophins (see Catt *et al.* *Nature* **280**, 109; 1979), it was thought logical that LHRH and its agonists exerted their inhibitory effects via the pituitary.

But in fact, there was already published

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evidence to show that LHRH agonists could have extra-pituitary effects, as Rippel and Johnson (*Proc. Soc. Exp. Biol. Med.* **152**, 432, 1976) had reported inhibition by an LHRH agonist of human chorionic gonadotrophin (hCG)-induced ovarian and uterine weight gain in hypophysectomized rats. This brief challenge to one of the bastions of endocrinological thinking seems to have gone largely unnoticed. But in 1979 the walls began to crumble when Hsueh and Erickson demonstrated inhibition by LHRH of the follicle-stimulating hormone (FSH)-induced increase in oestrogen and progesterone secretion by rat ovarian granulosa cells *in vitro* (*Science* **204**, 854, 1979), and followed this with a similar report concerning the male (*Nature* **281**, 66; 1979). In this they showed that concomitant treatment of hypophysectomized immature male rats with FSH and

LHRH agonist prevented the increases in testicular weight, LH-receptor numbers and steroidogenic responsiveness induced by the FSH treatment alone.

Confirmation of the extra-pituitary actions of LHRH and its agonists has been readily forthcoming. Ying and Guillemin (*Nature* **280**, 593; 1979) demonstrated a dose-dependent inhibitory effect of an LHRH agonist on gonadotrophin-induced ovarian weight gain in hypophysectomized female rats and also prevented ovulation, findings which a smaller study by Mayar *et al.* (*Proc. Soc. Exp. Biol. Med.* **161**, 216; 1979) confirmed. A preliminary report also suggests that LHRH agonists may directly influence ovum development following induction of super-ovulation (Erickson & Hsueh, *U.S.A. Soc. Gynec. Invest.* Abstract 371; 1980). In the male, Arimura and colleagues have shown that treatment with an LHRH agonist can reduce LH-receptor numbers in the testes of both immature and adult hypophysectomized rats (*Biochem. Biophys. Res. Commun.* **90**, 687; 1979). Outside of the gonads, inhibition by LHRH of progesterone production by human placental tissue *in vitro* has been reported (Wilson & Jawad *Fertil. Steril.* **33**, 91; 1980), and preliminary work in baboons by McRae, Vickery and Stevens (*Endocrinology Supp.* in the press), suggests that LHRH agonists can suppress chorionic gonadotrophin secretion by the placenta. However, such effects may not be straightforward, as LHRH agonists do not impair the placental secretion of hCG and progesterone *in vivo* in women (Casper *et al. Proc. of P.A.R.F.R. Workshop on Fertility Regulation* Harper & Row; 1980). Gonadal or placental effects of LHRH are presumably

important in explaining the local abortifacient action of LHRH on the pregnant rat uterus (Jones *Contraception* **20**, 569; 1979) and the termination by LHRH of pregnancy in rats hypophysectomized on day 12 (Bex & Corbin *61st. Endocr. Soc. Meeting, U.S.A.* Abstract 435; 1979).

The two most impressive aspects of the above findings are, firstly, that all but one of the extra-pituitary effects of LHRH and its agonists are inhibitory and, secondly, that the effects (particularly those on the gonads) are almost identical to those reported following similar treatment of intact animals. This raises the question as to whether all of the inhibitory effects of LHRH agonists in intact animals, including the contraceptive effects in women, are the result of extra-pituitary action? Although any answer must at this stage be qualified, the weight of evidence suggests this is not the case. To obtain the direct gonadal effects reported above, the investigators have used doses of LHRH agonists at least 10-times and, in most cases, over 1,000-times greater than those used in intact animals. Moreover, the direct effects reported have often been the inhibition of rather specialised changes which may not be directly relevant to the intact adult animal. Thus the acute steroidogenic responsiveness *in vitro* of Leydig cells from untreated rats is unaffected by LHRH or its agonists (Badger *et al. Endocrinology* **106**, 1149; 1980), although Clayton and colleagues (*Nature* **282**, 90; 1980) found a small decrease in the sensitivity of rat luteal cells to hCG-stimulation *in vitro* in the presence of 10^{-7} M LHRH agonist.

However, there is the very real possibility that some proportion of the inhibitory

effects of LHRH and its agonists in intact animals is the result of extra-pituitary action. Indeed, the recent demonstration of specific, high affinity ($K_A = 10^9 \text{M}^{-1}$) receptors for LHRH and its agonists on luteal cells in the ovary (see last ref. above), and on Leydig cells in the testis (Labrie *et al. Int. J. Fertil.* in the press; Sharpe & Fraser *Biochem. biophys. Res. Commun.* in the press), must mean that LHRH has some role to play at the gonadal level, particularly as these receptors have almost identical properties to LHRH receptors in the pituitary (see Clayton *et al. Endocrinology* **105**, 1369; 1979). There is also persuasive evidence that the direct effects of LHRH agonists on the gonads are mediated through such receptors, as Hsueh and Erickson (*Life Sci.* **25**, 1223; 1979) have shown that the inhibitory effects of LHRH agonists *in vitro* can be prevented in a dose-dependent manner by a structurally similar, but antagonistic, analogue of LHRH. There is also immunohistochemical evidence for LHRH receptors in mouse adrenal cells (Bernardo *et al. J. Histochem. Cytochem.* **26**, 613; 1978).

Because of the specialized portal blood system, which transports LHRH from the hypothalamus to the pituitary, and because of the rapid degradation of LHRH, it seems certain that LHRH secreted from hypothalamic neurones can never reach peripheral tissues in effective concentrations. Are there, therefore, extra-hypothalamic sites of LHRH production? Two pieces of information suggest that there are. First, there is some evidence for the placental production of LHRH (Khodr & Siler-Khodr *Science* **207**, 315; 1980), and secondly, Guillemin and colleagues



100 years ago

The *Times* Geneva correspondent writes under date June 20 that a remarkable electrical phenomenon occurred at Clarens on the afternoon of Thursday last. Heavy masses of rain-cloud hid from view the mountains which separate Fribourg from Montreux, but their summits were from time to time lit up by vivid flashes of lightning, and a heavy thunderstorm seemed to be raging in the valleys of the Avants and the Alliaz. No rain was falling near the lake, and the storm still appeared far off, when a tremendous peal of thunder shook the houses of Clarens and Tavel to their foundations. At the same instant a magnificent cherry-tree near the cemetery, measuring a metre in circumference, was struck by lightning. Some people who were working in a vineyard hard by saw the electric "fluid" play about a

little girl who had been gathering cherries and was already 30 paces from the tree. She was literally folded in a sheet of fire. The vine-dressers fled in terror from the spot. In the cemetery six persons, separated into three groups, none of them within 250 paces of the cherry-tree, were enveloped in a luminous cloud. They felt as if they were being struck in the face with hailstones of fine gravel, and when they touched each other sparks of electricity passed from their finger-ends. At the same time a column of fire was seen to descend in the direction of Chatelard, and it is averred that the electric fluid could be distinctly heard as it ran from point to point of the iron railing of a vault in the cemetery. The strangest part of the story is that neither the little girl, the people in the cemetery, nor the vine-dressers appear to have been hurt; the only inconvenience complained of being an unpleasant sensation in the joints, as if they had been violently twisted, a sensation which was felt with more or less acuteness for a few hours after. The explanation of this phenomenon is probably to be found in Prof. Colladon's theory of the way in which lightning descends, as described

in *Nature*, vol. xxii. p.65. The Professor contends that it falls in a shower, not in a perpendicular flash, and that it runs along branches of trees until it is all gathered in the trunk, which is bursts or tears open in its effort to reach the ground. In the instance in question the trunk of the cherry-tree is as completely shivered as if it had been exploded by a charge of dynamite.

Amongst the peculiar institutions of Paris are the street astronomers, who exhibit through their telescopes the moon, sun-spots, comets' tails, and other celestial objects, according to circumstances. Their charges vary from 1*d.* in the suburbs to 5*d.* on the Place de la Concorde or the Place Vendôme, where the instruments are not unworthy of a regular observatory. At the last monthly meeting of the scientific journalists M. Flammarion read an address sent to him by the corporation of these itinerant teachers of the marvels of the heavens. They state that from the beginning of the publication of the "*Astronomie Populaire*" the number of their customers has more than doubled.

from *Nature* **22**, 1 July, 1980.

(*Comptes Rendus* **D289**, 943; 1979; *Endocrinology* **106**, 114A; 1980) have identified a substance which is immunologically distinct from LHRH but has LHRH-like biological activity and is secreted by ovarian granulosa cell cultures and is present *in vivo* in follicular fluid.

The endocrine dogma concerning LHRH-like biological activity and is hypothalamic LHRH-producing neurones as unique and to view the portal blood supply as an insurance that LHRH reaches the pituitary without dilution in the peripheral circulation. The same was originally thought for somatostatin, which is also produced by hypothalamic neurones and acts on the pituitary, but is now known to be produced in the gut and elsewhere. It is therefore not too wild a speculation to consider LHRH as a 'transmitter' produced by nerve endings or other cells in the gonads or elsewhere. Certainly, if one accepts this possibility then the role of the portal blood system and the rapid

degradation of LHRH by the pituitary can be viewed not only as an efficient local transport system, but also as a means of preventing hypothalamic LHRH from reaching peripheral target tissues.

It seems appropriate to end on another note of speculation. Before the advent of the enzymically-resistant LHRH agonists, binding studies with LHRH were plagued by hormone degradation problems, but there were at least two reports of extra-pituitary binding sites specific for LHRH (Marshall *et al. Clin. Endocr.* **5**, 671; 1976 and Heber *et al. Amer. J. Physiol.* **235**, E227; 1978), which were dismissed as unimportant because of their low affinity ($K_A = 10^5 M^{-1}$). These sites included the ovary and testis, which we now know to contain high affinity receptors for LHRH. Can we therefore expect that the other tissues identified (liver, spleen, renal cortex, cerebral cortex, lung) will also prove to have high affinity receptors for LHRH and its agonists? □

How does the mantle move?

from Geoffrey F. Davies

WHEN the kinematic theory of plate tectonics was developed over a decade ago, two kinds of questions were posed. The first kind concerned the implications of moving plates of lithosphere for geological processes and for the interpretation of the geological record. The second kind of question concerned the involvement of the underlying mantle and the dynamics of the plate mantle system: how much of the mantle is involved, what is the pattern of flow in the mantle, and which of several possible types of driving buoyancy forces are dominant? Although many parts of the system are now more clearly understood, the way in which they fit together into a working machine is still unclear. This is only partly because of the inaccessibility of the mantle to direct observation: it is also because of the extremes of mechanical and thermal states in the plate-mantle system, which pose a considerable challenge to would-be modelers of the system. Some of the current attempts being made to understand the system were illustrated by papers presented at a recent conference*.

It is usually presumed that the plates and underlying mantle comprise a thermally convecting system driven by the earth's internal radiogenic heat, but mantle convection is very different from the more familiar examples of convection in the atmosphere or laboratory. This is because the mantle has a very high Prandtl number (so that viscous forces dominate over inertial forces) and its rheology is complex. The stress-strain rate relation of mantle minerals is strongly dependent on temperature, and is strongly non-linear at high stresses; also, high stress at low

temperatures can cause fracturing which will be observed as earthquakes. The Rayleigh number of the mantle is variously estimated to be between one million and one thousand million, which implies that temperature gradients are very large, notably near the top boundary. Combining the steep temperature gradient near the earth's surface with the temperature dependence of the rheology, one gets a picture of the lithosphere as a thermal boundary layer which is also a mechanical boundary layer: cold and therefore stronger, but also brittle. This rapid variation of temperature and mechanical properties in the lithosphere is an obstacle to finite-difference numerical modeling, since a prohibitive number of grid points is required. Also, the rheology of this lithosphere is not easily characterized in a manageable way. For example, simply increasing the viscosity in the lithosphere works for those parts of plates away from plate boundaries, but not near subduction zones, since brittle or quasi-plastic yielding may allow the large deformations associated with bending and subducting the lithosphere to occur at much lower stresses than would be predicted by a viscous rheology. Much of the recent work on plate-mantle dynamics has focussed on the lithosphere, either exploring and characterizing its rheology, or taking advantage of the order it imposes by being rigid most of the time.

The rheology of the lithosphere can be studied by using the constraints of bathymetric, gravity and geoid anomalies near subduction zones and volcanic seamounts. Models have evolved through simple elastic and viscous lithospheres to composites involving elastic-perfectly plastic or more complex rheologies. J. H. Bodine and A. B. Watts of Columbia

University presented results showing that the current subsidence of Hawaii relative to Oahu at the rate of 4.1 mm per yr can be explained as a delayed response of the lithosphere to the load imposed by Kohala volcano on Hawaii, which was active until about 0.4 Myr ago. In their model, the initial response of the lithosphere is that of a thick elastic layer, but on longer time scales (a million years or more) the lower, hotter part of the lithosphere deforms, allowing stress relaxation. The result is that after a million years or so the effective elastic thickness of the lithosphere is two or three times less than the initial affective thickness. A. Cazenave and B. Lago of Toulouse presented results of analogous models incorporating plastic or semi-empirical rheologies, and they predict that the relaxation time of the lithosphere is about five percent of the age of the lithosphere at the time it was loaded. A. B. Watts reviewed analyses of gravity and bathymetric anomalies by himself and his colleagues showing that the long-term effective elastic thickness of the lithosphere is a strong function of the age of the lithosphere at the time of loading. The results are consistent with the elastic thickness being proportional to the square-root of age, as is predicted by a simple cooling half-space model of the lithosphere. The same conclusion was reached by analysis of geoid anomalies derived from the GEOS 3 and SEASAT radar altimeters (M. C. Rousfousse, Smithsonian Astrophysical Observatory, Cambridge).

Although the lithosphere is difficult to incorporate into mantle convection models, its role as a rigid mechanical and thermal boundary layer has been exploited in several ways. P. Olson of Johns Hopkins University presented a generalization to spherical geometry of a boundary layer theory first applied to the mantle (Turcotte & Oxburgh *J. Fluid. Mech.* **28**, 29; 1967). Such a theory is ideally valid in the limit of high Rayleigh number, when the interior of the fluid is nearly isothermal and temperature gradients are concentrated in thin thermal boundary layers. Olson concluded that realistic surface velocities of several centimeters per year could be attained if convection was allowed to extend throughout the mantle (down to about half of the earth's radius), but could not if convection was confined to the upper 700 km of the mantle. However, Olson's models did not include a realistic lithosphere rheology, so this conclusion should probably be regarded as tentative at this stage.

Olson and several other speakers were concerned with testing a semi-empirical relation, based on laboratory and numerical experiments and on boundary layer theory, which states that the Nusselt number is proportional to the Rayleigh number to a small power, usually between

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*The joint Spring Meeting of the American Geophysical Union and several Canadian Geophysical Societies was held in Toronto.

one-fifth and one-third. The Nusselt number measures the efficiency of convective heat transport, and the relation has been applied to calculating thermal histories of planets (eg. Sharpe & Peltier *Geophys. J. R. astr. Soc.* **39**, 171; 1979; Reynolds & Cassen *Geophys. Res. Lett.* **6**, 121; 1979). E. R. Ivins and R. J. Phillips of the Jet Propulsion Laboratory, Pasadena, reported on numerical models with temperature-dependent viscosity in which the Nusselt number was systematically lower than in analogous constant viscosity cases. Again, their results may have been affected by an oversimplified lithospheric rheology, which did not include any stress dependence. E. M. Parmentier and J. Morgan, III of Brown University reported on numerical models which did include a moderately stress-dependent rheology. Their results implied that an initially hot, radioactively heated planet would approach a steady state about twice as fast as in the case of a stress-independent rheology, presumably because the larger buoyancy forces associated with higher temperatures would see a lower effective viscosity.

R. A. Lux and I. S. Sacks of the Carnegie Institution of Washington were concerned with the bottom boundary of the mantle, where seismologists have long suspected a

layer of irregularities one or two hundred kilometers thick. If there is significant heat flow from the core, then one would expect a boundary layer at the bottom of the mantle complementary to the lithosphere at the top: the bottom layer would have higher temperatures and lower viscosities. One might further expect such a layer to be subject to strong Rayleigh-Taylor instabilities, which might then be the source of the seismic heterogeneities. Lux and Sacks presented results of numerical models which showed that the lower viscosities in the boundary layer allow faster horizontal flow, and the resulting larger velocity gradients actually stabilize the layer, at least in their two-dimensional models. If the instability occurred, it would have to be in the form of rolls or sheets aligned with the flow.

The usefulness and versatility of the rigid boundary layer concept of plates was further illustrated by R. J. O'Connell of Harvard University and B. H. Hager of the State University of New York at Stony Brook, who used it to investigate the chemical stratification of the mantle and the effect of plate motions on sea level. In a joint paper, O'Connell and Hager noted that the migration velocities of ridges and trenches are not negligible compared with expected mantle flow velocities. A

consequence is that as trenches and ridges sweep around the globe, upper mantle material may be sampled more often than lower mantle material: roughly speaking, material recently subducted at a trench may be pulled back up by a passing ridge before it sinks to great depth. Thus a purely kinematic aspect of the plate-mantle system may give rise to a chemical gradation of the mantle, in which the shallower mantle is, on average, more strongly depleted in the incompatible elements which accumulate in continental crust.

Hager gave a new twist to an old problem by suggesting that changes in sea level relative to continents depend mainly on whether subducted lithosphere descends under continent or under ocean. Both the thermal contraction associated with the cold lithosphere and the deviatoric stresses transmitted from a sinking plate to the surface tend to lower the earth's surface above the plate. Thus subduction under continent would result in transgression and subduction under ocean would lead to regression. Hager estimated that the magnitude of this effect is considerably larger than that produced by changes in the volume of ridge systems (and hence of ocean basin volumes) resulting from changes in plate velocities. □

MHC restriction, self-tolerance and the thymus

from Jonathan Howard

THE phrase 'MHC restriction' was originally coined to describe a property of cytotoxic T lymphocytes, cells of the specific immune system which kill virus infected cells by a process involving direct membrane contact between the two cells. Specific receptors on the cytotoxic cell surface recognize and bind to virus-specific antigens on the membrane of the infected target cell. By an unknown mechanism this close contact results in lysis of the target. Oddly, however, the virus-specific antigens are not sufficient for the binding of cytotoxic cells: membrane molecules specified by the major histocompatibility complex (MHC) of the target cell are involved in the binding so intimately that they may be regarded as forming a component of the antigens recognized. In practice what this means is that cytotoxic T cells of a mouse of MHC type *A*, immunized with some virus *X*, will kill *X*-infected targets of MHC type *A* but not otherwise identical *X*-infected targets of MHC type *B*. That cytotoxic T cells recognize foreign antigens only in association with specific MHC antigens is the basic fact of MHC restriction. One can rationalize the involvement of membrane bound MHC molecules in this interaction by arguing that their presence in close association with virus specific molecules

enables cytotoxic T cells to identify and kill virus infected cells while ignoring an excess of free virus.

Since its first clear definition in 1974, MHC restriction has become recognized as a fundamental feature of antigen recognition by many (if not all) classes of T cell and seems to be a corollary of the fact that specific T cell activities are probably mediated, like target cell killing, by cell-cell contact. Other examples of such activities are T cell induction by exogenous antigen, involving MHC molecules on antigen presenting cell surfaces at the beginning of an immune response, and T cell help for antibody production by B cells, this latter activity probably being mediated through MHC molecules presented to T-helper cells by the B cell surface.

A key question is, where and when is the dual specificity of the observed response established? At what point is the discrimination between different MHC molecules and different foreign antigens made? Until relatively recently it would have been a good bet that the critical stage

for both discriminations was at the first encounter of a mature T cell with foreign antigen *X*. At this stage *X* is undoubtedly presented to the immune system by a specialized presenting cell which, in addition to trapping antigen, also carries MHC allelic products. If *X* and an MHC product are recognized together in some association by a subset of T cells, for example killer cell precursors carrying a receptor of the appropriate specificity, then the killer cells that are activated will kill only those targets which similarly express both the 'right' MHC allele and the 'right' *X*. Evidence in favour of this idea came from studies on the properties of T cells from F_1 hybrids between two strains carrying different MHC alleles, *A* and *B*. It was found that such animals carried two separate T cell populations, one able to recognize *X* + *A* and the other *X* + *B*. Normally both populations were immunized by *X*, but if *X* was experimentally presented to (*A* × *B*) F_1 T cells on cells of homozygous *A* origin, for example, only the *A* + *X* reactive subset was activated.

Of course in all such simple MHC restriction experiments another class of recognition process could be imagined where T cell activation required that MHC antigens on the reactive cell should 'match' MHC antigens on the antigen presenting cell or

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target. Such a like-like requirement amounting to self-recognition could be excluded by experiments in radiation chimaeras. Irradiated ($A \times B$)F₁ recipients were reconstituted with a mixture of marrow cells or foetal liver of homozygous A and B origins. T cells of A type grown up in such chimaeras could collaborate with B cells of B type for the production of antibody. Related experiments with cytotoxic T cells have also been done successfully. These results are perfectly compatible with the idea that the specificity of H-2 restriction is laid down at the moment of antigen presentation. If, therefore, 'self' recognition is involved in MHC restriction, T cells must 'learn' the MHC type of self during their ontogeny: self does not necessarily coincide with the MHC carried by the T cells themselves.

The idea that T cells in some way learn a range of possible MHC restrictions during their ontogeny before meeting antigens is now widely accepted (see *Nature* 272, 11; 1978). T cells differentiate from their precursors in the thymus gland and experiments by Bevan¹, Zinkernagel² and others gave results which suggested that some learning of MHC restriction specificity occurs during this maturation. Chimaeras were established in which the thymus was of MHC type A while the differentiating T cells were of F₁ type $A \times B$. When T cells from such $A \times B \rightarrow A$ chimaeras were immunized with a variety of antigens on $A \times B$ presenting cells the resultant immune response showed a marked bias towards restriction by the thymus H-2 type A . The unique capacity of the thymus to determine the restriction bias was demonstrated by experiments using thymus grafts^{4,5,6}.

A great deal of emphasis has been laid on this thymic 'bias'. It seems likely, however, that the results may have been greatly over-interpreted. If thymus learning of an MHC restriction repertoire were absolute, a T cell population restricted to MHC type B should never be found in animals in which $A \times B$ T cells had differentiated in an A thymus. In fact, however, some responses restricted to the B haplotype, albeit relatively weak, are often found^{5,7}, and immunization of such animals with antigens presented on cells of B type seems preferentially to immunize this B -restricted repertoire to give substantial responses⁸. There is a thymic MHC restriction bias, and it clearly requires an explanation. Nevertheless it is only a bias, and can be overridden by appropriate immunization.

Another class of experiment says even more clearly that 'learning' in the thymus cannot alone be responsible for determining the range of MHC restriction. These experiments involve analysis of the immunological reactivity of cells from normal A donors. The question is whether in any circumstances these cells can produce an MHC-restricted response to an antigen presented on cells carrying only MHC type B . Such experiments are com-

plicated by the fact that cells carrying MHC type B are strongly antigenic to normal T cells of A origin by virtue of the MHC incompatibility itself. This alloreactivity can, however, be removed from normal A T cell populations experimentally by a process resembling filtration. Cells from normal A donors are injected into the blood of irradiated $A \times B$ or B recipients. Alloreactive T cells from the donor are retained in the tissues of the recipient while cells unable to recognize the B alloantigens pass through the host tissues into the lymphatics from which they can be collected. Such 'negatively selected' populations can now be tested for their ability to recognize a foreign antigen X presented on cells of MHC type B in the absence of an alloreaction directed against the B alloantigens themselves. These experiments often, but not always, produce substantial responses restricted by B ⁹⁻¹². When they do, they demonstrate clearly that the MHC type of the thymus does not necessarily predict the MHC restriction repertoire of the T cells derived from it. In negative selection experiments the 'wrong' MHC type is not encountered by the responsive population at all during ontogeny, yet after appropriate antigen presentation a repertoire can be found for a response restricted by this MHC type.

What then, is the explanation for the thymic bias in chimaera experiments? For the time being this question must remain open. There is, however, some evidence to support the idea that the thymic bias may be maintained in chimaeras by suppression^{13,14}, probably acting on the repertoire at a peripheral rather than thymic level. It is as if responses to antigens presented by the non-thymic MHC type were actively inhibited. It is tempting to associate this kind of explanation for the thymic bias with the problems faced by $A \times B \rightarrow A$ chimaeras in the acquisition of self tolerance to their MHC alloantigens. One specialised function often proposed for the thymus is as a place in which autoreactive T cells generated by some process of repertoire diversification are eliminated. If this elimination is normally mediated by contact with antigens on the non-lymphoid cells of the thymus, $A \times B$ T cells differentiating in an A thymus cannot acquire tolerance of their own B MHC

antigens in the normal way. It has been shown in both mice and rats that very profound tolerance to MHC antigens absent from the fixed cells of the thymus, (as in neonatal A strain animals injected with $A \times B$ marrow at birth) may be mediated by specific suppressor cells^{15,16}. There is no demonstrated mechanism by which such suppressor cells work, but the effect is to prevent expansion of the anti- B reactive repertoire and the animal is effectively tolerant of B . The extension of these findings to $A \times B \rightarrow A$ chimaeras required for an explanation of the restriction bias towards A is to argue that the animals maintain a suppression of the alloreactive anti- B repertoire, and this suppression also includes exogenous antigens presented in association with the B allelic product. It would be of considerable interest to know whether suppressor cells from an A type donor made neonatally tolerant of B at birth can influence the restriction repertoire of an $A \times B$ recipient to mimic the thymic MHC restriction bias of an $A \times B \rightarrow A$ chimaera.

An extension of this argument is that in a normal A type animal there is no biological need for suppression of reactions restricted by MHC-type B . After removal of alloreactivity against B by filtration, as described above, A type T cell populations may therefore retain a repertoire able to recognize an antigen restricted by B more easily than do T cells from $A \times B \rightarrow A$ chimaeras. In experiments involving Vaccinia virus in mice Doherty and Bennick have demonstrated this effect quite clearly¹⁷. MHC type A T cells negatively selected and unreactive against B alloantigens can produce a good primary anti-Vaccinia virus response restricted by MHC type B , while cells from an $A \times B \rightarrow A$ chimaera do not. One can argue that in this case the postulated suppression of reactivity to B and B -associated antigens in the chimaera is more 'inclusive' than the depletion of reactivity to B by negative selection.

It is obviously time to re-evaluate the thymus learning experiments. Clearly something special happens in the thymus: the question is, what? I can think of three clearly distinct functions for the organ, firstly, the functional differentiation of T cells from immunologically incompetent precursors; secondly, the distribution of a diverse repertoire among separate clones of cells by repeated cell division; and thirdly, the elimination of autoreactive cells from that repertoire to induce self tolerance. Only the third process involves specific interference with the response repertoire. Although there may not be a fully consistent argument available, it is tempting to attribute the normal MHC restriction behaviour of T cells to the specificity of normal self tolerance induction in the thymus, and abnormal MHC restriction behaviour in $A \times B \rightarrow A$ chimaeras to an abnormal mechanism of induction of self tolerance in a non-thymic site. □

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ARTICLES

Pb–Sr–Nd isotopic correlation and the chemistry of the North Atlantic mantle

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The Pb, Sr, Nd isotopic composition in North Atlantic mid-oceanic ridge basalts has been measured. A positive correlation is found between Sr and Pb and a negative one between Nd and Sr. There is also a negative correlation between Hf/Th or Ta/Th and isotopic ratios such as $^{87}\text{Sr}/^{86}\text{Sr}$ or $^{206}\text{Pb}/^{204}\text{Pb}$. Using the data for oceanic island basalts with these coherent geochemical characteristics we discuss the origin of chemical variations in the North Atlantic mantle and the problem of the mantle geochemistry.

SINCE the discovery of the variation of Nd isotopes in the terrestrial mantle¹, it has been argued that this variation seems to be anticorrelated with Sr isotopes but not with Pb isotopes. This problem is well expressed by the Sr–Pb isotope ‘non-correlation’ plot established by Sun and Hanson^{2,3}. Thus Pb isotopes do not apparently follow the same logic as Nd and Sr isotopes in the mantle geochemistry and have been one of the problems in understanding mantle evolution. We have tried to clarify this problem by careful study of a restricted area and then by applying the results to other parts of the world. We also include a discussion of trace elements.

We have analysed tholeiites from 15 different sites of the Mid-Atlantic Ridge. Their locations are indicated on Fig. 1. The FAMOUS area has been studied in detail, and our results will be published elsewhere. We give here only mean or key samples from this area. We have measured Pb and Sr isotopic compositions on the same sample in each case with special treatment for Sr to minimize seawater exchanges. The positions, results and error limits are given in Table 1.

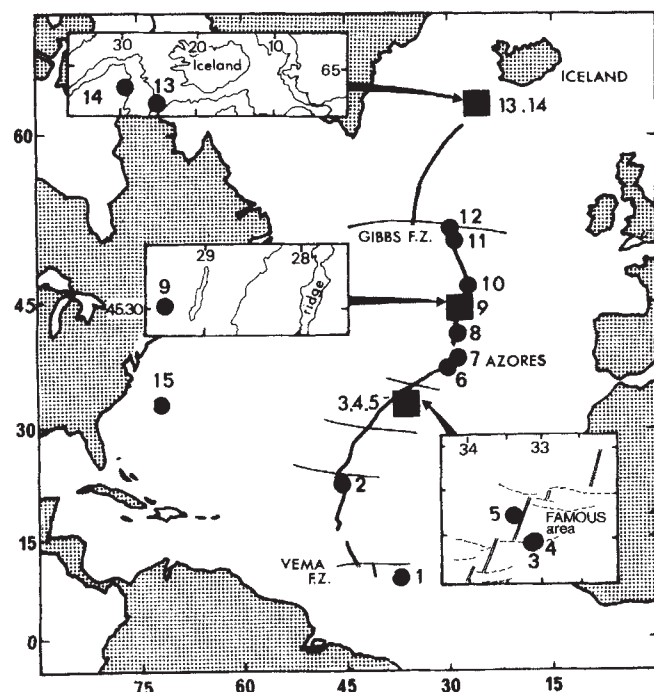


Fig. 1 Situation of the North Atlantic MORB samples.

Isotopic results

For the North Atlantic ocean, the expected inverse correlation between the Sr and Nd isotopic compositions is observed (see refs 1, 4, 5 and Fig. 2c). Comparison of the $^{206}\text{Pb}/^{204}\text{Pb}$ with $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ with $^{208}\text{Pb}/^{204}\text{Pb}$ diagrams give quite good correlations as was suggested by Tastumoto's work⁶ on Pb isotopes in oceanic ridge basalts (Fig. 2a).

In contrast, the direct correlation between the Pb isotopes and the Sr isotopes is less expected (Fig. 2b). An inverse correlation between Nd and Pb isotopes is, of course, also obtained. Such an observation is true for $^{206}\text{Pb}/^{204}\text{Pb}$ as well as for the other Pb isotopic ratios. Note that one sample¹⁰ is slightly out of correlation. When Sr isotopes were analysed for this sample by White and Schilling⁷ without any treatment (leaching or diopside separation) a $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70347 was obtained. Leaching lowers their value and as our leaching was incomplete our value may still be slightly too high, in which case the correlation could become perfect.

To appreciate this result one must remember that the Pb system on one hand and the Nd–Sr system on the other have been generally considered as being decoupled. This follows from Sun and Hanson's² plot in which a large cloud of points is observed rather than the line in the Nd–Sr diagram. We have then, for the first time, a coupling between Sr and Pb isotopes on mid-oceanic ridge basalts (MORB), which is exactly opposite to what has been claimed by Vidal and Dosso⁸. These remarkable regularities, also obtained by Cohen *et al.*⁹ but on samples from more dispersed geographical areas in different oceans, are the basis of our following discussion.

Assuming that the present day Nd isotopic composition of the closed mantle is equal to that of achondrites and chondrites, the corresponding values of $^{87}\text{Sr}/^{86}\text{Sr}$ and Rb/Sr ratios can be deduced from the correlation diagram of Nd–Sr. These values are 0.70475 and 0.03 (ref. 10) with large errors. Using the same procedure for the Pb isotopes with our Sr–Pb isotope correlation, where we have more data. We obtain the following values.

$$^{206}\text{Pb}/^{204}\text{Pb} = 21.3\text{--}22$$

$$^{207}\text{Pb}/^{204}\text{Pb} = 15.78\text{--}15.90$$

$$^{208}\text{Pb}/^{204}\text{Pb} = 41.5\text{--}42.3$$

These values are called the ‘planetary lead Atlantic’ values (PLA) because they correspond to the planetary Sr in the correlation diagrams. Application of these values to the classical $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$ diagram reveals immediately that the

Table 1 Location and Sr and Pb isotopic composition of the samples studied

Samples	Geographical coordinates	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$	$^{87}\text{Sr}/^{86}\text{Sr}$	
AD5.5	9°39'N 40°27'W	18.709 18.670 0.015	15.508 15.496 0.017	38.16 38.12 0.05	0.70270 0.00004	
Leg 45 395A 33-2 9-13 9-13	22°N Leaching for lead	18.279 0.014 18.203 0.014	15.467 0.016 15.469 0.016	37.65 0.05 37.61 0.05	0.70233	2
395A 49-2 13-17	22°N	18.211 0.019	15.476 0.020	37.62 0.06		
Leg 49 hole 413	36°32.6'N 33°10.5'W 2,608 m	18.706 0.025	15.524 0.022	38.41 0.07		3
Leg 49 hole 412 7 2-5	36°33'74"N 33°09'96"W 2,609 m	18.965 0.012	15.555 0.015	38.52 0.05		4
412 11 32-35	36°33'74"N 33°09'96"W 2,609 m	18.949 0.015	15.579 0.017	38.42 0.05	0.70299 0.00006	
Leg 49 hole 411 2 12-13	36°45'97"N 33°23'30"W 1,935 m	18.844 0.015	15.559 0.016	38.29 0.05		5
DR 17	38°N 31°50'W	19.261 0.022	15.603 0.018	39.04 0.06	0.70307 0.00008	6
DR 24	39°N 30°W	19.444 0.018	15.587 0.018	38.98 0.06	0.70315 0.00007	7
TR 122 3D4	40°13'9"N 29°35.4'W 2,675 m	18.253 0.015	15.502 0.018	38.08 0.05	0.70299 0.00009 0.70298 0.00007	8
	Leaching for lead	18.381 0.024	15.515 0.024	38.19 0.07	strong leaching 0.70298 0.00007	
Leg 49 hole 410 41	45°30.51'N 29°28'56"N 2,975 m	19.730 0.018	15.619 0.019	39.70 0.06	0.70346 0.00004	9
55-60						
TR 138 1AD	46°31'6"N 27°29.1'W 2,400-2,550 m Leaching for lead	18.355 0.017 18.319 0.021	15.483 0.021 15.447 0.025	38.51 0.07 38.43 0.07	0.70316 0.00009	10
TR 138 6D1 glass whole rock	50°02'6"N 28°56.0'W 3,360-3,380 m 50°02'6"N 28°56.0'W 3,360-3,380 m	17.826 0.021 17.798 0.012	15.412 0.021 15.420 0.015	37.36 0.065 37.33 0.05	0.70231 0.00006	11
Gibbs Fracture zone D1M	52°59'6"N 34°58'7"W 1,875 m	18.24 0.09	15.49 0.09	37.9 0.2		12
Leg 49 hole 409 17 35-40	62°36'98"N 25°57'17"W 832 m	18.790 0.015	15.516 0.016	38.22 0.05	0.70299 0.00006	13
Leg 49 hole 407 42 87-89	63°56'52"N 30°34'56"W 2,472 m	19.058 0.02	15.528 0.023	38.43 0.07		14
Leg 52 Jurassic 15-3		18.559 0.018 18.483 0.015	15.488 0.020 15.506 0.016	37.76 0.06 37.79 0.05		15

The values obtained by Tatsumoto⁴⁰ on AD 5.5. are reported; all the others are our results. Mattinson⁴¹ measured Pb isotopes in some samples from the Leg 49 sites. We have obtained good agreement with his results. The Pb, Sr and Nd compositions have been measured using the techniques described respectively by Manhès *et al.*²⁵, Birck and C. J. A.³⁷ and Richard *et al.*¹ We have used the leaching technique on all samples for the Sr isotope compositions and also for some Pb measurements.

PLA values do not correspond to the closed system reference geochron (Fig. 2a), but are clearly in the so-called (*J*) field. This indicates that the U-Pb system has had an open system evolution through geological time. As, by definition, a planetary value should correspond to a closed system situation, the PLA values are certainly not planetary in the same way as Sr or Nd.

Relationship with trace elements

Trace elements are generally more difficult to handle than isotopic ratios because their concentrations are modified by magmatic processes (partial melting + fractional

crystallization + other processes such as seawater interaction). Some elements are strongly enriched into the liquid during magmatic processes and are unmodified by interaction with seawater. Therefore their ratios can be considered representative of their mantle source. Such elements, called H elements by Treuil and Joron¹¹, are Hf, Ta, Th, La, Zr, Nb. We have a set of values for some of them in the North Atlantic published by Joron *et al.*¹² and Wood *et al.*^{13,14}. Here we try to use only these elements, to avoid the secondary effects which would obscure the picture.

Sr and Pb isotopes have been compared with some H element ratios on Fig. 3a). Even if only five different sites can be used for

H elements, an inverse correlation is obtained between Sr or Pb isotopes and ratio like Hf/Th, La/Th, Ta/Th. The values obtained by Wood *et al.*¹⁴ on samples from 63°N (site 13) seems anomalous as the ratios vary strongly within the site. If the Hf/Th, La/Th, Ta/Th ratios are considered which correspond to the $^{87}\text{Sr}/^{86}\text{Sr}$ planetary value a set of values is obtained which are very different from the chondritic values. For example Hf/Th corresponding to $^{87}\text{Sr}/^{86}\text{Sr} = 0.70475$ is about 1, while the chondritic value is about 10.

In a plot of Hf/Th versus Ta/Th where the ratios have the same denominator, mixing processes are represented by a straight line, as in the lead isotopes plot. The most remarkable observation (Fig. 3b) regarding the results for the North-Atlantic MORB is that they do not define simple straight lines. Therefore the variation for the North-Atlantic MORB source cannot be generated by a simple two-end member mixing process. Such a conclusion agrees with that of Langmuir *et al.*¹⁶ which was obtained using different elements.

The 'regional' variations

A cross-section of regional variations along the ridge can be made from Iceland to the South. With our new Pb and Sr data which have been corrected for seawater contamination by a HCl leaching technique, we can draw two important conclusions from the regional variations which have been a source of confusion in the past.

(1) There is no clear correlation between bathymetry and isotopic ratios. Certainly some correlation occurs between the depth of the ridge and some trace element contents, especially for the incompatible ones, but this probably reflects a correlation between the bathymetry and the degree of partial melting rather than the composition of the mantle source. On the North-Atlantic scale, there is no clear correlation between bathymetry and isotopic ratios which would directly reflect the nature of the mantle source.

(2) The variation from Iceland to the South is not dominated by only two 'hot spots' (Iceland + Azores). Our Pb, Sr and Nd isotopic results reinforce those of White and Schilling⁷ on Sr isotopic composition because Pb and Nd are not sensitive to alteration. The variations are such that if one wants to interpret the variations of isotopic ratios by a 'hot spot' model, a third hot spot must be added around 45°N and perhaps another around 9°N.

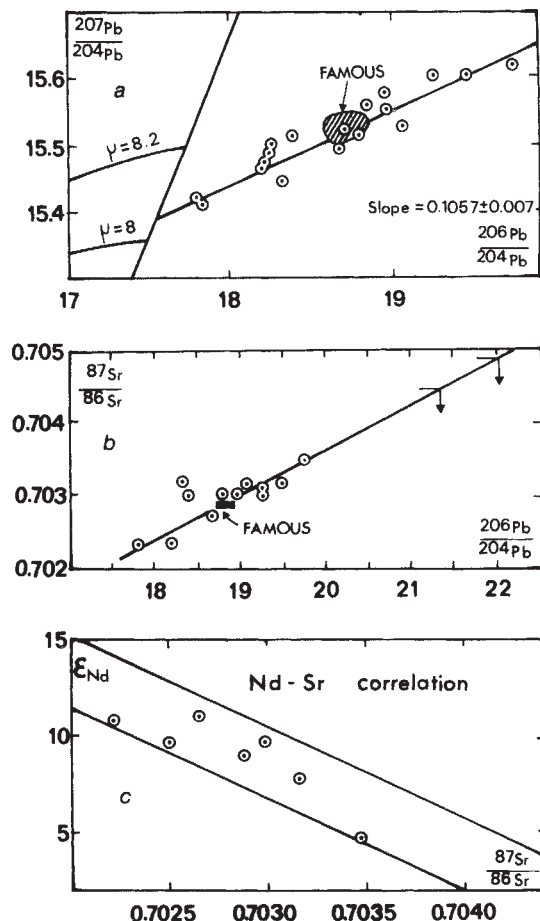


Fig. 2 Results for samples from North Atlantic tholeiites. a, $^{207}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$. The regression line has a slope of 0.1057 ± 0.007 . The model age is 1,720 Myr. b, Correlation $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ for North Atlantic MORB. Lead isotopic Atlantic planetary values are determined from $^{87}\text{Sr}/^{86}\text{Sr}$ planetary value (0.70475). c, Nd-Sr isotopes correlation for the North Atlantic basalts. Data for MORB are from refs 1, 4, 5.

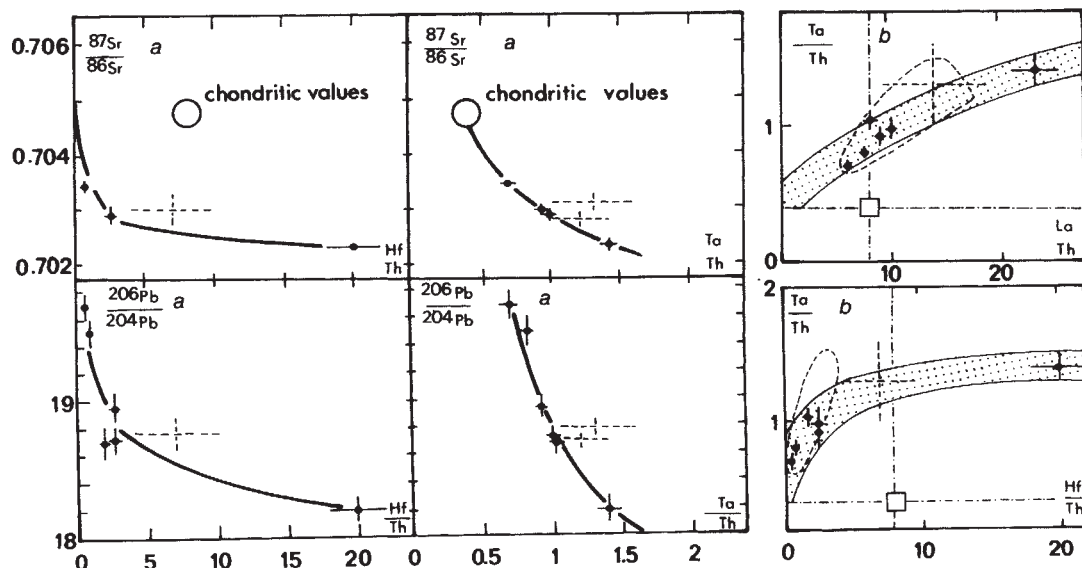


Fig. 3 a, $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ versus Hf/Th or La/Th trends for North Atlantic MORB. Data are from refs 12-14 and this paper (chondritic values are from refs 42, 43). The Hf/Th and La/Th ratios are the mean value of these ratios for each site. The error of 0.1% for the isotopic ratios is taken as being equivalent to the dispersion observed on the FAMOUS site where many samples have been measured³⁸. b, Ta/Th versus Hf/Th or La/Th trend for North Atlantic MORB. (Refs as in Fig. 4.) The dashed line area corresponds to oceanic islands domain (data from ref. 21).

Comparison of the North Atlantic MORB with other oceanic basalts

Only a few results have been obtained on the same samples for Pb–Sr–Nd isotopes (Fig. 4) using modern techniques and leaching or diopside separation to eliminate Sr seawater contamination. The only available results are those of Cohen *et al.*⁹ on volcanic glasses and some of our unpublished results on Pacific and Indian oceans. Pb–Pb or Sr–Pb diagrams show a very good positive correlation which is identical with our Atlantic lines. Thus the values for Pb isotopes corresponding to the planetary strontium isotopic ratio are about the same as PLA. This being so the above anomaly should be considered a worldwide characteristic and not a local one.

We do not have measurements for some other oceans but preliminary data for the Pacific MORB seem to plot on the general trend in the Hf/Th–Ta/Th diagrams (H. Bougault, personal communication).

As Pb, Sr and Nd isotopes have been analysed in Icelandic samples^{17–20} and in the Azores islands (see ref. 15 for Pb; refs 1, 22 for Sr and Nd) we can then try to place these samples with respect to MORB in the (Sr, Pb) isotope correlation diagram.

Iceland, Terceira, Corvo and Santa Maria are roughly in the correlation band. Other islands, like Faial or Sao Miguel are out of the trend. Sao Miguel is also outside the (Nd–Sr) main trend²². However, a more careful analysis shows that two populations of oceanic island basalts which do not follow the trend are older than the others. For instance, only the old part of the Sao Miguel island does not really follow the trend. All other islands, including the Canary Islands²⁴, plot around the main trend. Of course, this argument is rather statistical but the positive correlation including the North Atlantic islands is clear.

Although different isotopic ratios were often found in separate samples we will use the compilation of Sun³ (Fig. 4). Such a compilation shows that the basalts from oceanic islands define a large band completely outside the MORB (Pb–Sr) or (Pb–Pb) correlations. In the (Pb–Sr) correlation diagram such a band can be even considered 'orthogonal' to the MORB's trend. In the (Pb–Pb) diagram, there is also a clear distinction between these two populations. Such a behaviour is in great contrast with the (Sr–Nd) diagram, which exhibits the correlation for both MORB and OIB.

We also want to emphasize another major difference. In the Sr–Nd diagram, the values observed for OIB can be interpreted as the result of a material derived from a planetary reservoir and contaminated by the MORB reservoir during its ascent. In contrast, for Pb isotopes, the OIB reservoir is clearly in the (*J*) type field, and thus corresponds to a largely open system (independent of any contamination by the MORB reservoir). Thus the OIB reservoir is certainly not the protected closed system proposed by Wasserburg and De Paolo²⁶.

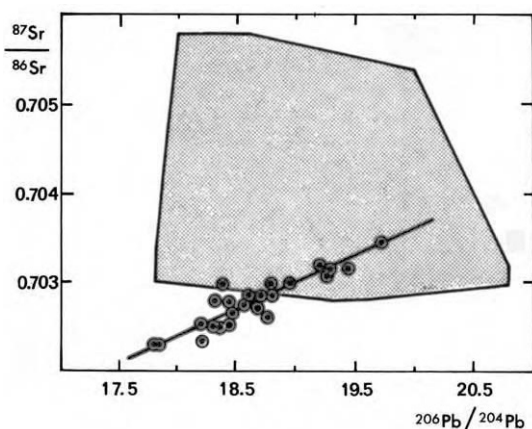


Fig. 4 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ for oceanic basalts. Oceanic island basalts do not seem to be correlated (dotted area from ref. 3) whereas MORB data define a positive correlation from our data and those of Cohen *et al.*⁹

We have plotted a large number of data concerning oceanic islands in (Hf/Th, Ta/Th) and (La/Th, Ta/Th) diagrams (Fig. 3b) which show that Ta/Th ratios from MORB and OIB, although distinct are very similar, and not too far from chondritic values. In contrast Hf/Th and Hf/Ta ratios are much lower in OIB than in MORB, and are very different from chondritic ratios. Unfortunately as Hf is less hygromatophile than Ta, Th and La (ref. 11) and because alkali basalts are created by small degrees of partial melting (with perhaps more or less fluid transfer and metasomatism), one does not know whether the Hf/Th and Hf/Ta ratios for OIB reflect that of their source or result from their formation processes. Thus we shall not include these ratios in the discussion of OIB sources.

Theoretical interpretations

The main result of the previous investigations is that we clearly have a coherent behaviour of the Rb–Sr, Sm–Nd, U–Th–Pb and Hf, Ta, Th, La systems for the North Atlantic province. This is the first demonstration of such a coherence with such detail. We will now try to explain the significance of these trends.

Models can be built in which the mantle heterogeneity is created by liquid–solid fractionation processes operating through geological time²⁷. In this context, the inverse correlation between Nd and Sr isotopes, and the $^{87}\text{Sr}/^{86}\text{Sr}$ depletion compared with planetary values can be easily interpreted²⁷. These observations can be understood if one remembers that Rb is enriched in liquid compared with Sr, and that Nd is enriched in liquid compared with Sr.

If the U–Th–Pb system is taken into account in this model, the positive correlation between Pb and Sr isotopes indicates that U and Th are more enriched in magmatic liquids than Pb. This conclusion agrees with the observations made in magmatic suites²⁸ and apparently solves the Pb paradox previously pointed out by C. J. A.²⁹. However, as we have mentioned above, the problem with Pb is that the 'planetary values' determined from the correlations are not on the Pb–Pb closed system curve.

In the same way, the negative correlation between Hf/Th, Ta/Th, or La/Th and $^{87}\text{Sr}/^{86}\text{Sr}$ correspond to the fact that Hf, Ta or La are less enriched in magmatic liquids than Th. The slopes of the correlation lines in Hf/Th–Ta/Th and Hf/Th–La/Th diagrams indicate the degree of relative enrichment of Hf, Ta and La in the liquid. Then we can deduce the following increasing order of enrichment in the liquid: $\text{Hf} < \text{La} < \text{Ta} < \text{Th}$, which corresponds more or less to what can be established from the observation of the trace element fractionation in magmatic suites¹¹.

The preceding observations lead us to distinguish, on a worldwide scale, the existence of two reservoirs, one for MORB, one for OIB. Such a distinction was in fact made in the first isotopic measurements on MORB by Tatsumoto⁶ and was subsequently adopted by others^{2,30}. However, we feel that evidence presented here reinforces such an interpretation. Our description of these two reservoirs differs from the usual one. We do not consider here the subcontinental lithosphere which constitutes another reservoir, and this is discussed elsewhere³¹.

The MORB reservoir is called the upper mantle, the OIB reservoir is considered to be the lower mantle. The OIB is probably partly contaminated by the MORB reservoir during ascent.

The upper mantle is isotopically extremely coherent but not homogeneous. The data for the Atlantic as well as the Pacific or Indian basalts (ref. 9 and our unpublished data) clearly indicate that variations may occur on regional scales. For trace elements, such variations are even more pronounced compared with the lower mantle. The variations observed in MORB are not the result of contamination by OIB as is usually claimed, but are created by the geodynamical system linked with the seafloor spreading which implies chemical fractionation. Imperfect homogenization results from convection. The basis of such processes which can be called 'chemical geodynamics' are discussed elsewhere^{27,36}.

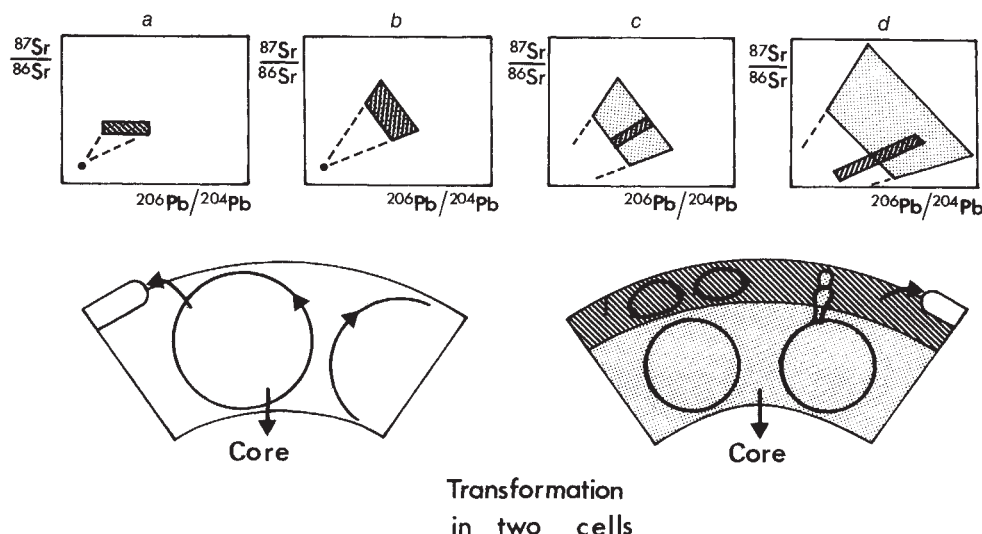


Fig. 5 *a*, Fractionation of U/Pb ratios in the early history of the Earth by core formation creates variations of Pb isotopic ratio only. *b*, The formation of the continental crust fractionates both Rb/Sr and U/Pb ratios, creating variation of Sr and Pb isotopic ratios. *c*, *d*, The transformation of the whole mantle into two independent cells leads to the creation of two types of reservoirs with distinct isotopic characters: relatively homogeneous and less radiogenic MORB reservoir compared with a heterogeneous and more radiogenic OIB reservoir.

The lower mantle is the source for OIB but is not an homogenized, uniform reservoir. It may be more uniform for Sr, Nd and H elements than the upper mantle, but strongly heterogeneous for Pb isotopes and then U/Pb ratios. This reservoir is not planetary for Pb isotopes, for some H elements, and probably also for Sr or Nd as has been shown for Sao Miguel²². This means that the reservoir has been fractionated in some way.

The different behaviour of Pb isotopes compared with Sr isotopes is illustrated by the lack of Pb–Sr correlation for OIB and by the planetary Atlantic values being in the *J*-field in the Pb–Pb diagram. There have been two interpretations for these features:

(1) An interaction between the core and the lower mantle has been admitted through geological times; Pb is buried in the core because it is chalcophilic and the core contains sulphide. The rate of burying is assumed to decrease exponentially with time, with a time constant of 5,000 myr. This mechanism had been previously considered for a short period just after the differentiation of the Earth^{32,33}. Vidal and Dosso⁸ have also discussed this hypothesis but in their model, Rb was also buried; in such a case the Nd–Sr correlation would be destroyed, which does not occur. Although this long-term interaction has not been proposed by geochemists it agrees with the suggestion by Runcorn³⁴ of a continuous, but decaying with time, growth curve for the core.

(2) The alternative interpretation proposed by O'Nions *et al.*³⁵ allows a reinjection of sediments into the lower mantle. These sediments are H elements enriched by upwards migration within the continental crust. Reinjection rate decreases with time. However, the similarity of hygromagmatophile element ratios such as Ta/Th and La/Th for MORB and OIB does not favour such a model. Furthermore, this model would decouple Sr and Nd isotopes but not Pb and Sr as it is U and Rb simultaneous enrichment of the upper continental crust which is observed.

Note that a fundamental consequence of our observations is that the two reservoirs were connected at some time in the past.

The Pb isotopes systematic of MORB clearly show that the PLA values are anomalous. One way of explaining such anomalies is by assuming that the upper mantle exchanged with the core in the past. Some convection between upper mantle and core is needed. In the same way, H elements in the lower mantle should have been fractionated through time as their values are not chondritic. These elements have no chalcophile or siderophile character and the interaction with the core has no effect on them. The method of fractionating such elements is related to

liquid–solid fractionation (or CO₂–magma fractionation?) or in other words to processes associated with the surface activity. This also leads to the conclusion that the two reservoirs were once linked.

Hence we conclude that convection cells affected the whole mantle during the Archaean and maybe even later. During that time seafloor spreading and continental crust differentiation occurred at the same time as the core–mantle interaction. Later, the convection cells broke up and split in two cells, or continuously differentiated an upper mantle. The exact process for this mechanism remains unclear. In the lower cells, only the U–Pb system fractionated further, as probably did all other siderophile and chalcophile elements. The upper cells, probably of smaller dimension, are fractionated further through continental crust formation and vigorously stirred by advection. This creates the presently observed patterns of isotopic variations in MORB. And indeed, ~2,000 Myr are necessary to create the observed variation for Pb, Sr and Nd isotopes by such a process (Fig. 5). In which case a separated upper mantle would be an old phenomenon.

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Palaeomagnetic polarity in a 930-m core from Searles Valley, California

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Palaeomagnetic polarity to the base of the Mammoth subchron (3.15 Myr) has been determined in a core of lacustrine sediments from ancient Searles Lake. The magnetostratigraphy provides a chronological framework for palynological, tephrochronological and palaeoecological studies of the core. Five zones of inclination are found that are not in the polarity time scale of Mankinen and Dalrymple. Three of the zones may correlate with K-Ar-dated lava flows of that polarity elsewhere, and the other two may be new discoveries.

A 930-m, surface-to-bedrock core from Searles Valley, California (35°43'N, 122°40'E), has been used to study palaeoclimate in the western US¹. The upper 695 m is sediment from Searles Lake, now dry; the remaining 235 m is arkose deposited by intermittent streams on an alluvial fan. The history of climate in the Searles Lake deposits, the mineralogy and chemistry of which varied with the growth and recession of the lake². The presence or absence of the lake closely mirrored changes in climate, for Searles Lake was near the terminus of a chain of lakes fed by runoff from the Sierra Nevada³. As such, Searles Lake was perennial and deep during wet (pluvial) periods; during dry (interpluvial) periods like the present, the lake shrank to a playa or a salt flat.

A useful interpretation of palaeoclimate must be keyed to an accurate time base, which is also a requirement of corollary investigations (palynology, tephrochronology, palaeoecology), in progress, on the core. Radiocarbon dating of the upper ~50 m of valley fill is extensive⁴⁻⁷, but radiometric dating of sediment beyond the range of ¹⁴C has not been successful. The palaeomagnetic data reported here provide the necessary geochronology for the older portion of the core.

Searles Valley core

The core, called KM-3, was drilled in 1968 by the Kerr-McGee Chemical Corporation, which mines subsurface brines in Searles Valley, and in 1976, KM-3 was released to the US Geological Survey for scientific study. The core has a 9-cm diameter, and because of cracking during drying, is in pieces no longer than 50 cm each. However, some edges may be matched so that longer pieces can be reconstructed, some approaching 2 m in length.

Relatively thin, alternating layers of mud and salines make up the intervals between depths of 0–38, 69–228 and 291–403 m. Thick sections of deep-lake muds lie between depths of 38–69, 228–291 and 542–693 m. The interval between 403 and 542 m is brown sediment formed mostly in a playa lake¹. (Conforming to accepted terminology^{1,2}, we use 'salines' for the evaporite minerals, and 'mud' as a general term for clay, silt, and marl that was moist and plastic when extruded as core; now dry, the clay and silt are claystone and siltstone, respectively.)

There are numerous tephra layers in the core, and a sequence of them in the interval 169.3–178.6 m may represent the Bishop Tuff (R. Hay, personal communication), a pyroclastic erupted in Long Valley (California) ~0.7 Myr ago^{8,9}. A more positive identification of the tuff would provide an important time-line in the core, and this is in progress. Two other tephra layers lie near the bottom of the lacustrine section (684.0 and 690.9 m); if they too can be traced to their source and dated, another cross-check on the magnetostratigraphy will be available.

Sampling and laboratory procedures

Palaeomagnetic samples were taken at intervals of ~3 m or less in non-saline portions of the core below 100 m depth. Above 100 m, the core is not sufficiently intact to be reliable for palaeomagnetism. The large diameter of the core allowed us to take three specimens at each measured horizon. Each specimen was encased in a polythylene box of 6.6 cm³, and the specimens from a horizon had the same relative azimuth.

For each subset of specimens, we partially demagnetized one specimen in stepwise increments to 300 Oe peak alternating fields or higher, and demagnetized the other two at the median destructive field. This was 200–300 Oe (Fig. 1) for about 80% of the specimens; for the remaining specimens (from saline-rich horizons), the median destructive field was 50–150 Oe. Within most subsets, and almost always below 400 m where the remanence is nearly 100-fold stronger than that of saline-rich zones, the grouping of magnetic vectors is good—the spread of values of inclination being commonly <5°. Samples taken at 581.0 and 619.8 m (brown mud deposited in a playa) were thermally demagnetized, and the directions were compared with those of the a.f. demagnetized specimens from those depths; in each case, there was no appreciable change in the magnetic vector (Fig. 1).

Magnetostratigraphy in Searles Valley

Interpretation of polarity change in KM-3 is most convincing if linked to one or more reliable markers in the Pleistocene. Although only tentatively identified in the core, the Bishop Tuff would serve as one such calibration point because it lies near the

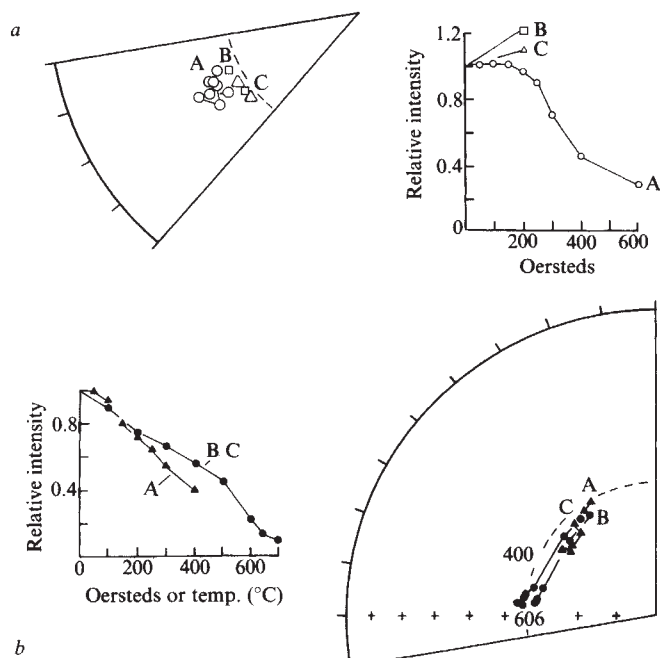


Fig. 1 Normalized a.f. and/or thermal demagnetization curves and equal-area plot of palaeomagnetic directions for: *a*, three specimens from 480.5 m (reversed polarity); and *b*, from 581.0 m (normal polarity). The azimuth of specimens within each horizon is the same, but different between horizons. In *b*, specimen A was a.f. demagnetized; specimens B and C were thermally demagnetized. The dashed line in the equal-area plot represents inclination of an axial dipole field (55°) for the site latitude; solid symbols are plotted on the lower hemisphere, open symbols on the upper

Brunhes/Matuyama boundary¹⁰. Indeed, the youngest horizon of negative inclination is at 195.0 m, or ~15 m below the zone of tephra layers that is thought to represent the Bishop Tuff. The nearest overlying horizon of positive inclination is at 174.9 m, so we interpret the Brunhes/Matuyama boundary at 185.0 ± 10.0 m (Table 1). The underlying ~320 m is predominantly of negative inclination (Fig. 2), which we assume to represent the Matuyama chron. Below that, the predominantly positive inclination is most probably the upper part of the Gauss chron.

Assuming that we have correctly identified the Brunhes/Matuyama and Matuyama/Gauss boundaries, it is then possible to plot age against depth to identify the zones of positive inclination within the Matuyama chron. From Fig. 3 it can be readily ascertained that the zones centred at ~210 m, 390 m, 410 m, and 450 m correspond to the Jaramillo, Olduvai, and two-part Réunion subchrons, respectively. Further, the zones of negative inclination in the Gauss chron that are centred at about 630 m and 660 m would correlate to the Kaena and Mammoth subchrons, respectively (Table 1).

Note in Fig. 3 that there is a nearly linear relationship between the position of the polarity boundaries and the age assigned to them using the time scale of Mankinen and Dalrymple¹¹. This is especially true for sediment below 400 m, where the lack of saline layers allows an approximation of uniform sedimentation rate. For those 295 m, spanning nearly 1.2 Myr, the average rate of sedimentation is ~ 40 yr cm^{-1} (0.25 mm yr^{-1}). It is difficult to calculate sedimentation rates for other parts of the core because the time represented by saline layers can be either very long or short; salines initially accumulate more rapidly than mud, but this is countered by periods of almost no deposition when the lake becomes a seasonally flooded salt flat². Nonetheless, the overall sedimentation rate to 695 m is ~ 45 yr cm^{-1} , or only 10% slower than that of mud of the perennial lake.

Five zones of inclination in KM-3 are not in the standard polarity time scale proposed by Mankinen and Dalrymple¹¹. Three of the zones may correspond to subchrons that have been proposed¹²⁻¹⁴ but not universally accepted, while the other two

could be new. These previously undetected short subchrons are not entirely surprising as their existence was predicted on the assumption that durations of polarity changes conform to a Poisson distribution¹⁵. The advantage of using lacustrine deposits such as in Searles Valley to search for short subchrons is that the sedimentation rate is at least 50-fold greater than in most deep-sea sediments, and is much less discontinuous than the eruption of lava flows. An obvious disadvantage is that part of the core might be inverted, accidentally either during initial recovery or some time during the intervening 8 yr of storage. As we were not present at the time of drilling, there is no guarantee that the top of the core was properly labelled on the drill rig or after splitting and storage; a realistic approach would be to assume that part of so long a core was inverted by human error. However, this probably did not occur during storage because we have compared the present core to Kerr-McGee's colour photographs of it taken at the time of recovery. When we removed samples, we photographed the potentially useful boxes of core, and those photographs have enabled us to check the orientation of back-up samples.

The question of inverted samples becomes critical when comparing measured zones of constant inclination to the generally accepted polarity time scale, or when detection of a new subchron is claimed. We examined notes made at the time of sampling to see if any of the unnamed short zones of inclination are restricted to a single core box or coring run, as these portions could be those most likely to be inverted. Three new zones of inclination were found restricted to single coring runs, and resampling of cores from adjacent runs showed that inversion of the core was probably not responsible for the zones. Additional checks included matching saw marks on the split side of the core to see if they were similar from piece to piece in a storage box, and determining whether the switch in inclination occurred between segments of core where the lithology suggested a discontinuity.

Matuyama chron

The zone of positive inclination that we interpret to be the Jaramillo subchron (0.90–0.97 Myr) lies between ~205 and 220 m (Table 1). It includes four measured horizons and spans two boxes of core. The two horizons of negative inclination between the top of the zone and where we place the Brunhes/Matuyama boundary (Fig. 2) are also from two boxes of

Table 1 Location of polarity boundaries and unnamed zones of inclination in KM-3

Boundary	Depth (m)	Lithology
Brunhes–Matuyama	185.0 ± 10.0	Interbedded mud and salines
Matuyama–Jaramillo	204.9 ± 0.1	
Jaramillo–Matuyama	221.2 ± 10.6	
Matuyama–Unnamed zone (N3)	281.1 ± 0.4	Mud (deep lake)
Unnamed zone (N3)–Matuyama	287.7 ± 2.1	
Matuyama–Unnamed zone (N2)	322.3 ± 2.5	Interbedded mud and salines
Unnamed zone (N2)–Matuyama	330.2 ± 1.7	
Matuyama–Olduvai	378.5 ± 1.1	Mud (playa)
Olduvai–Matuyama	398.9 ± 0.4	
Matuyama–Réunion*	408.3 ± 4.2	
Réunion–Matuyama	424.7 ± 1.7	
Matuyama–Réunion	447.4 ± 1.6	
Réunion–Matuyama	456.0 ± 4.6	Mud (deep lake)
Matuyama–Unnamed zone (N1)	516.5 ± 1.5	
Unnamed zone (N1)–Matuyama	519.8 ± 1.9	
Matuyama–Gauss	522.9 ± 1.2	
Gauss–Unnamed zone (R2)	569.3 ± 6.7	
Unnamed zone (R2)–Gauss	580.8 ± 0.3	
Gauss–Unnamed zone (R1)	599.8 ± 0.6	
Unnamed zone (R1)–Gauss	603.3 ± 1.4	
Gauss–Kaena	616.2 ± 1.5	
Kaena–Gauss	643.1 ± 2.6	
Gauss–Mammoth	651.7 ± 2.2	
Mammoth–Gauss	683.9 ± 4.2	

* We assume the Réunion to be a multiple subchron^{29,30}

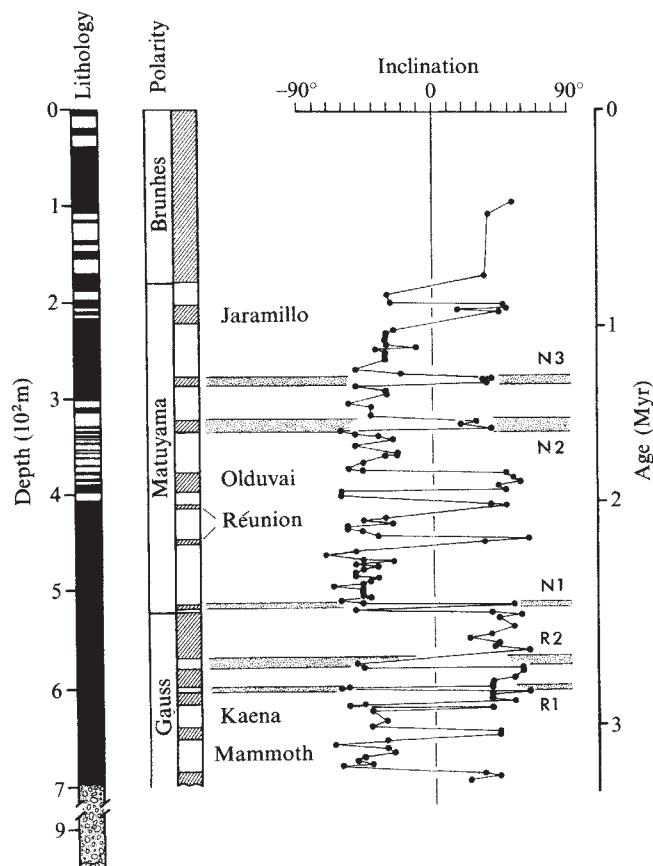


Fig. 2 Palaeomagnetic polarity in Searles Valley core KM-3. Shaded region highlights the unnamed zones of inclination, three in the Matuyama chron and two in the Gauss chron. In the lithological column: unshaded area, salines; black area, mud; spotted area, arkose. In the polarity column; hatched area, normal polarity; unshaded area, reversed polarity. Age is extrapolated from polarity boundaries in the time scale of Mankinen and Dalrymple¹¹, and by assuming a uniform sedimentation rate. Note that the time scale is not linear.

core, and the horizon of negative inclination below the zone is from another box. The switch from positive to negative inclination at the top of the zone occurs within one section of core pipe (each section is 10 m long), eliminating the possibility that the coring run was inverted or mislabelled at the time of recovery.

The other universally recognized subchron in the Matuyama chron is the Olduvai subchron (1.67–1.87 Myr); we place it between 379 and 399 m (Table 1). This portion of the core involves four measured horizons, one each from two different boxes of core and two from a third box. The third box also contains the horizon of negative inclination at the base of the subchron, and the two samples in that box are separated by ~0.3 m. Again, the change from positive to negative inclination occurs within one section of core pipe.

The two short zones of positive inclination below the Olduvai subchron we consider to be the multiple Réunion subchron (2.12–2.14 and 2.01–2.04 Myr)^{16–18}. The older half occurs between 447 and 456 m (Table 1) and includes two measured horizons, one each from separate boxes of core that is little disturbed. The samples of negative inclination that bracket the zone are from two other boxes of core, and the switch in inclination is within a section of core. The younger half is between 408 and 424 m, and the two samples came from the same box of core. As is the case for the older part of the subchron, the horizons of negative inclination on either side came from different boxes of core. However, unlike the older part, the positive inclinations are all in one section of drill pipe that was obtained separately from the sections containing the horizons above and below; this raises the possibility of an inverted section of core.

Unnamed zones of inclination in the Matuyama chron

The palaeomagnetic record in KM-3 contains three other short segments of positive inclination that are not included in the Mankinen and Dalrymple time scale¹¹. The youngest (N3; Fig. 2) is in deep-lake mud between 281 and 288 m (Table 1). The zone is found in two boxes of very good core from the same core run (the measured horizons above and below the zone are from different runs) and terminates 60 m below the start of the Jaramillo subchron; 54 of these 60 m are deep-lake mud. Using the 40 yr cm⁻¹ accumulation rate cited above for the mud, this part of the core spans ~20,000 yr between 1.24 and 1.26 Myr ago. If no time is assigned to the 6 m of salines between the disappearance of the deep-water lake and onset of the Jaramillo subchron, this unnamed zone is dated 1.20–1.22 Myr.

Mankinen *et al.*¹² recently reviewed the evidence for a short subchron close to and preceding the Jaramillo subchron, and have identified one (Cobb Mountain subchron) in the volcanic units near Clear Lake, California. The K–Ar age given the Cobb Mountain subchron is 1.12 ± 0.02 Myr, so we cannot closely match the unnamed zone of inclination in KM-3 with that one, but given the uncertainty in sedimentation rate, the correlation is possible. It might also equate with the K–Ar-dated basalt of normal polarity in Madiera¹⁹ and in the Lesser Antilles (Guadeloupe and St Vincent)²⁰ if the dates are accurate, and to a normally magnetized ash layer in the Osaka Group, Japan, having a fission-track age 1.1 ± 0.1 Myr (refs 21–23).

The zone of positive inclination near the middle of the Matuyama subchron (N2; Fig. 2) may correlate with the controversial Gilsa subchron, whose existence has been affirmed and denied several times during the past decade²⁴. The three measured horizons are in different boxes of core, and the samples used to bracket the zone are from two other boxes. Although the quality of that portion of the core is good, the horizons in question are from a single core run, and the opposite inclination on either side is from two other runs. Thus, we are concerned about whether the entire core run was mislabeled or inverted.

The oldest zone of positive inclination in the Matuyama chron (N1; Fig. 2) is near its beginning and might correspond to the 'X' subchron¹⁴ that has been seen in some magnetic anomaly data; however, it may correlate to the short subchron observed in Icelandic lavas very close to the Matuyama/Gauss boundary²⁵. The measured horizon is in part of the good core, and the switch in inclination occurs within one core run.

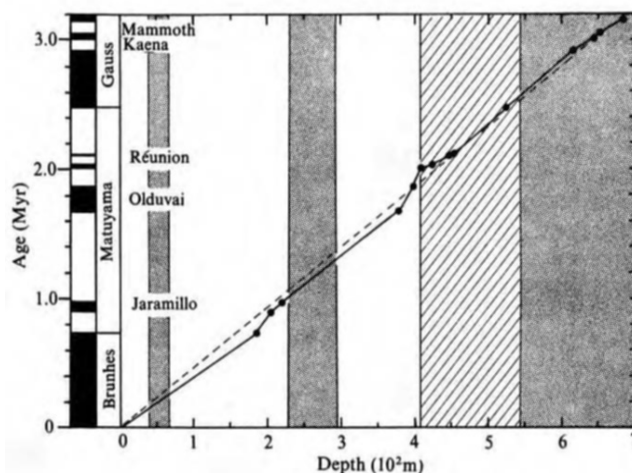


Fig. 3 Depth of interpreted polarity boundaries in KM-3 versus age in the polarity time scale¹¹. Shaded area, mud deposited by a deep, perennial lake; hatched area, mud deposited by a playa lake². Overall rate of sedimentation is ~45 cm yr⁻¹ (dashed line); for the perennial lake, it is 40 cm yr⁻¹.

Gauss chron

We equate the two zones of negative inclination near the bottom of the lacustrine section with the Kaena subchron (2.92–3.01 Myr) and Mammoth subchron (3.05–3.15 Myr). Each represents at least five measured horizons, and they are in different boxes of core. The two horizons of positive inclination that separate the subchrons are also from separate boxes of core, and the switch of inclination occurs in one core run.

Unnamed zones of inclination in the Gauss chron

Reversed magnetozones occur in upper Gauss time in mud from a perennial, deep lake, the sediment type we believe to be the most reliable magnetic recorder in the core. Using estimated sedimentation rates again, the younger zone (R2; Fig. 2) spans ~40,000 yr, and the older one (R1; Fig. 2) perhaps 20,000 yr. Extrapolating from the Matuyama–Gauss boundary (2.48 Myr) (ref. 11) the ages for these zones are then 2.67–2.71 and 2.79–2.81 Myr, respectively. Some reversely magnetized K–Ar-dated basalt flows in this age range have been reported: two from Oahu in Hawaii²⁶, one in west Iceland²⁷ and one in St Vincent²⁰. Thus, these may be real subchrons, but because of their brevity, they can only be clearly defined in a high sedimentation-rate environment such as Searles Lake. We must re-emphasize that the measured horizons defining both zones were found in single boxes of core (although the opposite polarity occurs in single core runs). For the younger of the two zones, there are two horizons of positive inclination and two of negative inclination, and the separation between the portion of the core

where the inclination switches is 0.46 m, or slightly less than 1,000 yr using the assumed sedimentation rate for that part of the core. Three closely spaced horizons of good core define the older zone, and the horizon above and below are in different boxes of core and at least 2.2 m apart.

Conclusions

Based on the record of palaeomagnetic polarity in KM-3, the oldest lake deposits in Searles Valley formed ~3.2 Myr ago (Fig. 2). The timing of a changing climate that might have produced a permanent lake in that part of the Great Basin coincides with a general cooling in the Northern Hemisphere as seen in the $\delta^{18}\text{O}$ record in deep-sea core V28-179 (ref. 28). The sedimentary record is probably a result of the interplay of tectonics and climatic events¹.

Five unnamed zones of inclination are present in the core, and four involve two or more measured horizons. The three unnamed zones in the Matuyama chron may correspond to subchrons that have been proposed by others but are not universally accepted. The two unnamed zones in the Gauss chron occur in good-quality core, and the change from positive to negative inclination for both zones occurs in single, 10-m sections of core.

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Nerve growth factors in the rat iris

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Nerve growth factor (NGF) activity was not detected by bioassay in irides killed immediately after excision but NGF appeared within 24 h in living irides placed in culture or grafted to a host eye. Furthermore, sensory and, although less effective, sympathetic denervation of irides in situ led within 10 days to the appearance of NGF activity. In addition, freezing and thawing released a parasympathetic neuronotrophic factor activity from irides.

THE protein nerve growth factor (NGF)¹ has been suggested to mediate trophic influences to sympathetic and sensory neurones from innervated target organs². However, little is known about the distribution of NGF in peripheral tissues to support this concept³. Except for some very rich sources of NGF (the male mouse submandibular gland¹, the guinea pig prostate⁴), mam-

malian tissues are normally inactive when tested as homogenates in the sensitive bioassay based on NGF-susceptible embryonic ganglia^{3,5}. We now show that explants of the adult rat iris immediately killed by freezing do not contain NGF at levels detectable by bioassay but that NGF is readily detectable in irides killed by freezing within a day after transfer to a recon-

stituted collagen gel⁶ (compare refs 7, 8) or to the anterior eye chamber of another rat⁹. After re-innervation of the iris transplants, NGF was no longer detectable. Most compelling is the fact that the levels of NGF were also raised in irides *in situ* 10 days after sensory denervation and, although less prominent, 10 days after sympathetic denervation. Furthermore, evidence was obtained for the actions of a parasympathetic, non-NGF, trophic factor released from the rat iris after freezing and thawing. The results support a role for NGF as a signal initiating and directing regenerating sensory and sympathetic fibres in adult mammals and suggest that other trophic factors may fulfill a similar role in other fibre systems.

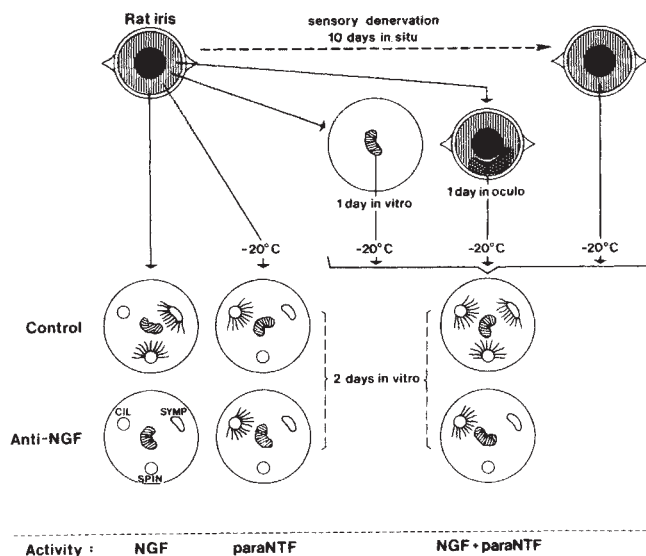


Fig. 1 Schematic representation of the experiments with rat irides and the resulting patterns of neurite growth responses in chick sympathetic (symp), spinal (spin) and ciliary (cil) ganglia. The results are interpreted as evoked by the actions of nerve growth factor (NGF), of a parasympathetic neuronotrophic factor (paraNTF) and of the simultaneous action of these two. Circles represent culture dishes. Irises were from normal rats (left) or from rats in which the trigeminal nerve had been lesioned (sensory denervation, right). For bioassay sympathetic, spinal and ciliary ganglia from 8- to 10-day-old chick embryos were placed surrounding a living or killed (by freezing to -20°C) half-iris in an 0.3–0.5-mm thick collagen gel in a 35-mm plastic culture dish. The medium of the gel was Eagle's basal medium supplemented with 1% fetal calf serum, penicillin (100 units ml^{-1}) and streptomycin ($100\text{ }\mu\text{g ml}^{-1}$). Parallel dishes received in addition 2–5 μl per ml of specific rabbit anti-mouse- βNGF antiserum²², levels that readily blocked the activity of 1 biological unit (BU) of NGF. Cultures were incubated at 37°C with 5% CO_2 in water-saturated air for 2 days and then examined by phase-contrast microscopy.

Effects of culturing on NGF synthesis in the iris

To study the content of NGF, irides of adult Sprague–Dawley rats were excised aseptically and tested in a ganglionic bioassay (Fig. 1). The criterion for NGF activity is an induction of neurite outgrowth in the chick sympathetic and spinal ganglia, which in parallel dishes can be blocked by antiserum to NGF, combined with a lack of fibre response in the ciliary ganglion⁶. Three distinct patterns of neurite outgrowth responses were observed (Fig. 1). Living iris explants taken directly into co-culture with ganglia evoked neurite outgrowth in the sympathetic and spinal ganglia within 20–24 h. Outgrowths developed fully within 2 days (Table 1), were directed mainly towards the living iris explants and had fibre densities resembling those evoked by 0.8 to 1 biological units of NGF (BU)¹. Anti-NGF nearly completely inhibited these outgrowths (Table 1, $P < 0.01$). Thus it is evident that the living irides in culture release NGF. The ciliary ganglia showed nearly no nerve fibre outgrowth when combined with living iris tissue. In control cultures without irides all three types of ganglia failed to extend neurites⁶ (Table 1).

To test whether NGF is present in freshly excised irides or whether it is synthesized *in vitro*, irides were killed by immediate freezing to -20°C for 1 to 30 days and then thawed. No NGF activity was detected in such iris tissue in the bioassay (Figs 1, 2a): in sympathetic ganglia slightly more fibres extended than in controls ($P < 0.05$) but anti-NGF did not significantly (by the *U*-test) reduce this outgrowth (Table 1). On the other hand, killed iris tissue significantly increased formation of neurite bundles in the ciliary ganglia (Fig. 2b; Table 1, $P < 0.01$). This stimulation was not abolished by adding anti-NGF, although the antiserum sometimes slightly impaired the response in the ciliary ganglion in this and in the following culture combinations. These results suggest the existence in the iris of a parasympathetic neuronotrophic factor² (paraNTF), stimulating the cholinergic neurones of the ciliary ganglion, and being released only after disrupting cells by freezing and thawing. Furthermore, the results show that NGF is not present at detectable levels in the iris *in situ*, but that the iris begins to synthesize NGF when placed in culture.

Effects of freezing and transplantation

To rule out the possibility that the freezing–thawing itself destroys NGF activity in the iris, explanted irides were cultured for 1 day in order to accumulate NGF before being killed. Bioassay demonstrated the same level of NGF activity in these explants as in the living ones (Figs 1, 2c; Table 1). Moreover, the ciliary ganglia exhibited dense outgrowths of neurites not significantly different from those evoked by immediately killed irides (Table 1). Thus these cultures showed a pattern of neurite stimulation that evidently represents the combined activities of NGF and paraNTF (Fig. 1). Anti-NGF inhibited outgrowth of neurites in the sympathetic (Table 1; $P < 0.01$) and spinal ganglia but did not affect outgrowth in ciliary ganglia.

Table 1 Relative density of neurite outgrowths in chick ganglia

	Without iris explants	With living iris explants	No special treatment	With irides killed after:			
				Culture <i>in vitro</i> (1 day)	Grafting <i>in oculo</i> (1 day)	Sympathetic denervation <i>in situ</i> (10 days)	Sensory denervation <i>in situ</i> (10 days)
Sympathetic ganglia without anti-NGF	0.0 $\pm$ 0.0 (8)	1.0 $\pm$ 0.0 (9)	0.3 $\pm$ 0.1 (9)	1.0 $\pm$ 0.0 (9)	1.0 $\pm$ 0.0 (5)	0.6 $\pm$ 0.1 (10)	0.9 $\pm$ 0.0 (15)
Sympathetic ganglia with anti-NGF	0.0 $\pm$ 0.0 (8)	0.1 $\pm$ 0.0 (8)	0.2 $\pm$ 0.1 (8)	0.4 $\pm$ 0.1 (5)	0.4 $\pm$ 0.1 (5)	0.3 $\pm$ 0.1 (7)	0.3 $\pm$ 0.1 (11)
Ciliary ganglia	0.0 $\pm$ 0.0 (8)	0.1 $\pm$ 0.1 (5)	0.7 $\pm$ 0.0 (5)	0.8 $\pm$ 0.1 (5)	0.8 $\pm$ 0.1 (5)	0.8 $\pm$ 0.0 (5)	0.7 $\pm$ 0.1 (13)

Mean values $\pm$ s.e.m. are given. For each group 5 to 15 ganglia (number given in parentheses) were scored for density of outgrowing neurites after 2 days on a blind basis. The scores ranged from 0 (no neurites at all) to 1 (maximum density, fibre halo with circular outline). The scores for the two types of ganglia are not strictly comparable due to differences in neurite morphology. *P* values given in the text refer to rankings of the individual scores evaluated by the Mann–Whitney *U*-test.

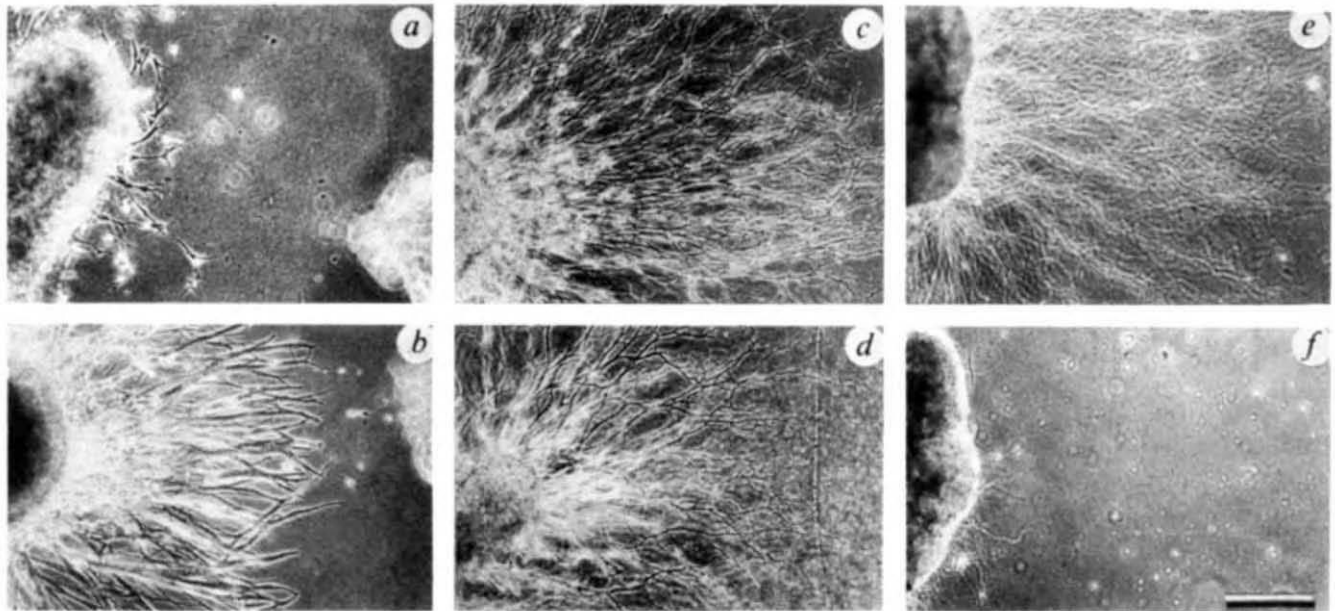


Fig. 2 *a*, Sympathetic ganglion (left) combined for 2 days in culture with rat iris (right) immediately killed after extirpation. No neurites emerge from the ganglion. *b*, Ciliary ganglion in the same conditions. In this parasympathetic ganglion extensive formation of thick neurite fascicles (identity controlled by the transmission electron microscope) is elicited by the killed iris explant. *c*, Sympathetic ganglion with dense fibre outgrowth elicited by an iris that had been cultured for 1 day and then frozen-thawed before being tested in the bioassay. *d*, Sympathetic ganglion combined with an iris transplanted to the anterior eye chamber of another rat for 1 day before being killed and tested. *e*, Extensive neurite formation in a sympathetic ganglion evoked by an iris killed 10 days after sensory denervation *in situ*. *f*, Inhibition by anti- β NGF of the neurite outgrowth in the foregoing combination. Phase contrast micrographs. Scale bar, 0.2 mm. See also ref. 13 for low-power darkfield micrographs of total outgrowths elicited by living irides.

To test whether NGF is produced in the iris only in *in vitro* conditions, the iris was either transplanted to the anterior eye chamber of another rat, rather than to a culture dish, or was left *in situ* but subjected either to sympathetic denervation by extirpation of the superior cervical ganglion or to sensory denervation by lesioning the trigeminal nerve⁹ (Fig. 1). To prevent appearance of NGF during the subsequent bioassay the irides were killed by freezing before being combined with ganglia (Fig. 1).

After 1 day in the anterior eye chamber the transplanted iris contained the same amount of NGF as iris explants cultured *in vitro* for 1 day (Fig. 2*d*; Table 1). After 4, 7 and 11 days *in oculo* the same levels of NGF activity persisted but after 1 month, at which time the iris transplant had become at least sympathetically reinnervated by the host iris¹⁰, NGF activity was no longer detectable. The responses evoked in the ciliary ganglia were the same as those elicited by the other frozen-thawed irides (Table 1).

Role of innervation in NGF synthesis

Experiments with irides denervated *in situ* show more clearly that the level of NGF is in some way related to the state of innervation. Irises taken for bioassay 10 days after sensory denervation consistently gave dense fibre outgrowths in sympathetic (Figs 1, 2*e*, Table 1; the increase over normal killed irides is significant, $P < 0.01$) and spinal ganglia. The resulting outgrowth could be significantly reduced by anti-NGF (Figs 1, 2*f*, Table 1; $P < 0.01$). Sympathetic denervation for 10 days resulted in a much less although significant ($P < 0.05$) increase in fibre density in sympathetic ganglia (Table 1). That NGF is involved here also is indicated by the significant ($P < 0.05$) reduction in fibre outgrowth by anti-NGF (Table 1). No NGF was detected 2 or 4 days after denervating the iris *in situ* nor was NGF found 1 month after denervation (at which time some reinnervation is likely to have taken place). Combined sensory and sympathetic denervations gave as high levels of NGF after 10 days as did sensory denervation. For the ciliary ganglia the

denervations caused no further increase in fibre density above that evoked by normal killed irides (Table 1).

Sections of the ganglia cut for light microscopy at the end of bioassay add some information on the survival of neurones in response to the irides. In full agreement with the neurite outgrowth response the ciliary ganglion showed excellent neuronal survival after exposure to any iris that had been frozen-thawed (Fig. 3*a*; 19 ± 1 neurone profiles were found within an area of 0.01 mm^2 ; mean $\pm$ s.e.m. from sections of 5 ganglia) whereas living irides to a less ($P < 0.01$) extent did promote survival of ciliary neurones (Fig. 3*b*; 5 ± 1 neurone profiles with living irides compared with 1 ± 0 neurone profiles in cultured control ganglia, $P < 0.05$). The outcome for sympathetic ganglia was less expected: here the presence of iris tissue enhanced neuronal survival whether or not outgrowth of neurites occurred (Fig. 3*c*). The number of neurone profiles was thus 63 ± 4 with normal killed irides, which is not significantly different from the 71 ± 4 neurone profiles found with killed irides plus anti-NGF, whereas neuronal survival was significantly lower ($P < 0.01$) in cultured control ganglia (Fig. 3*d*; 30 ± 3 neurone profiles). The enhancement may be due to an nonspecific, non-NGF, trophic influence on the neurones exerted by the presence of serum in the explanted irides: thus adding rat serum at 10% by volume resulted in 56 ± 5 neurone profiles. Rat serum, however, totally failed to induce neurite outgrowth in the sympathetic as well as the ciliary ganglia.

The present experiments show that the level of NGF may be raised in a mammalian tissue *in situ* by sensory or sympathetic denervation. The results thus favour the concept that a tissue after denervation during adulthood, and probably also during development, signals with NGF to initiate and attract growing or regrowing sympathetic and sensory nerve fibres^{1,2}. This view is also supported by the finding that NGF content falls to undetectable levels concomitant with reinnervation of the iris grafts.

The appearance of NGF in the iris as a result of explantation, transplantation or denervation may be the result of an active

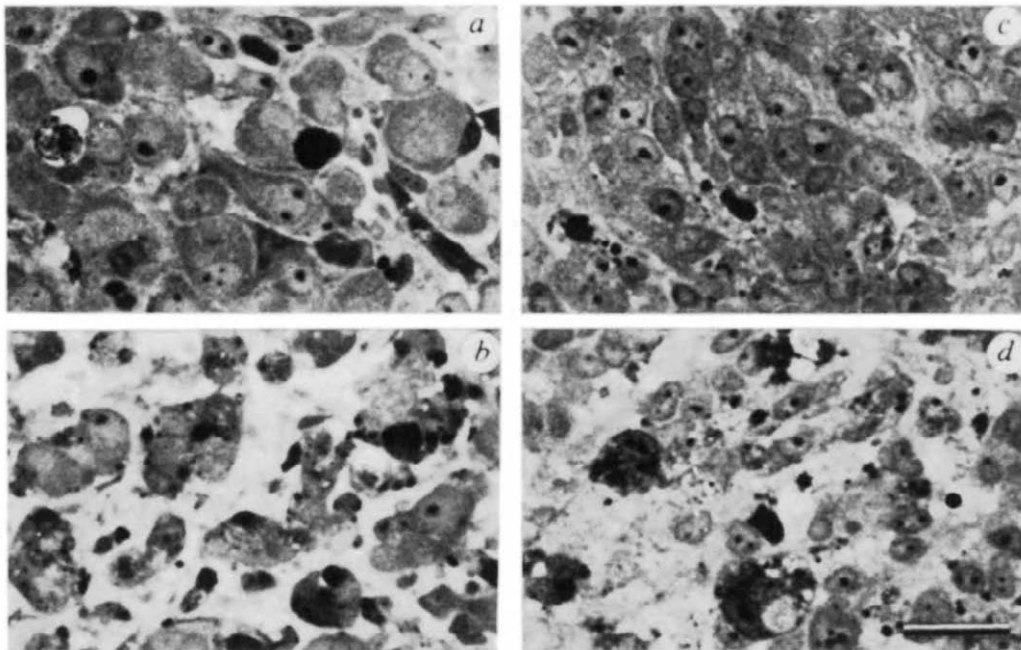


Fig. 3 *a*, Ciliary ganglion combined with a killed rat iris for 2 days in culture. A high neuronal survival is evident. *b*, Same but with a living iris explant. A massive death of neurones, also seen in control cultures, is not prevented by the living iris. *c*, Sympathetic ganglion cultured with an iris killed immediately after extirpation. Despite the addition of anti- β NGF and the fact that no or only few neurites extend from the ganglion (see Fig. 2*a*), a markedly good neuronal survival is induced by the iris tissue. *d*, Sympathetic control ganglion cultured without the presence of iris tissue. Only a few neurones survive in these conditions. For data on neurone survival, see text. Micrographs of toluidine blue-stained 2- μ m sections of ganglia fixed in 1.5% glutaraldehyde plus 1.5% paraformaldehyde and embedded in Epon. Scale bar, 20 μ m.

response of the iris to increase the production of the factor. Although less plausible, it may also be that the iris is normally drained of the NGF produced in it by retrograde axonal transport¹¹ to such an extent that the appearance of NGF after, for example, denervation represents an accumulation of the factor rather than an increased production.

The amounts of NGF released from the iris explants that induce a 0.8 to 1 BU response in chick ganglia (Fig. 2*c, d, e*) can be roughly calculated. The response was evoked only within a zone of about 2 mm around the approximately 5 $\times$ 1 mm half iris in the 0.3–0.5-mm thick gel. Thus the half iris spreads NGF to about 10–20 μ l of the gel. If it is assumed¹ that 1 BU corresponds to 5 ng of NGF per ml and that the NGF is evenly distributed, this volume should contain about 40–80 pg. The wet weight of the half iris is about 0.5 mg and thus a reasonable amount of NGF released is of the order of 80–160 ng per g wet weight of cultured or denervated iris tissue. This value is slightly higher than that stated by Johnson *et al.*⁸ measuring the release of NGF from irides to a culture medium by radioimmunoassay (RIA). Given the limits of detection of NGF activity for the present bioassay (0.03–0.06 BU)⁶, and as we did not detect any NGF activity in initially killed irides, this tissue should release less than about 5–10 ng of NGF per g wet weight. Johnson *et al.*⁸ stated that the iris initially contains 15–20 ng NGF per g as measured by RIA but this may be an overestimation due to their technique¹². The present results thus show that the level of NGF is increased by at least one order of magnitude in the positive iris explants.

It is noteworthy that sensory denervation was a much more potent stimulus for the NGF increase *in situ* than sympathetic denervation (Table 1). One possible explanation for this difference may be that the lesioning technique used to obtain sensory denervation also damages a considerable proportion of the sympathetic axons to the iris. This may also explain why a

combined sensory and sympathetic denervation was not found more effective in stimulating the production of NGF than was the trigeminal lesion alone. It is also of note that sensory denervation has been shown to cause massive ingrowth of central noradrenergic fibres into the iris from transplants of nucleus locus coeruleus (see ref. 9). Since this nucleus does not respond to NGF¹³ the mechanism for this fibre stimulation is, however, unlikely to be a response to the increase in NGF demonstrated here.

A parasympathetic stimulation was evident by irides treated by freezing and thawing, indicating the presence in the iris of trophic factors besides NGF. Similar trophic factors have been found in rat cardiac and skeletal muscle^{5,14}. Factors stimulating the ciliary ganglion have also recently been reported in the embryonic chick heart^{6,15,16} and chick iris^{17,18} as well as in culture medium conditioned by chick heart¹⁹ and muscle cells²⁰.

Conclusions

Thus two nerve growth stimulating factors, one affecting sympathetic and sensory neurones and the other affecting parasympathetic neurones, were found in rat iris tissue. The present results provide the first demonstration that the level of NGF is raised in a tissue *in situ* as a result of denervation. Our data are in line with the demonstration that immunosympathectomy¹ is brought about by neutralizing endogenous NGF²¹ and support the concept of NGF as a messenger between target organs and peripheral neurones in mammals.

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Comparison of white pock (h) mutants of monkeypox virus with parental monkeypox and with variola-like viruses isolated from animals

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Monkeypox mutants arising spontaneously or after serial, high multiplicity passage were characterized phenotypically and by restriction endonuclease mapping. Some resemble 'whitepox' and variola viruses in several of the markers tested but all are distinguishable phenotypically from these. None resembles 'whitepox' viruses in genome structure although near-terminal deletions or symmetrical, terminal rearrangements, relative to parental monkeypox, occurred. 'Whitepox' viruses isolated from animals closely resemble variola in both phenotype and genome structure.

THE last naturally occurring case of smallpox was in Somalia in 1977 and the WHO is satisfied that a human reservoir of this disease no longer exists. Attention is now focused on whether there exists any non-human reservoir of variola virus that might threaten the permanence of the eradication of smallpox. A few cases clinically resembling smallpox have occurred sporadically in villages or small towns in the rain forest belt of West Africa. These were caused, not by variola virus, but by another pox virus called monkeypox¹. Monkeypox virus has shown no evidence of sustained transmission in man and seems not to be a serious public health problem².

Attempts to find the natural reservoir of monkeypox virus in West Africa have so far failed to provide any isolates of this virus from wild animals. Four pox viruses, however, which have been recovered from specimens of animals caught in Zaire, have proved indistinguishable from variola virus by biological tests in the laboratory. These four and two similar viruses isolated from monkeys imported into Holland from Malaysia have collectively been called the whitepox viruses. Their natural history is unknown, but there are two reports^{3,4} that viruses with the laboratory phenotype of whitepox virus have been obtained from stocks of monkeypox virus, either from hamsters inoculated with monkeypox or by cloning on the chick chorioallantois (CAM).

The implication is that monkeypox virus, which does on rare occasions infect humans, can also produce, as a genetic variant, a virus which may have the same propensities as variola. It is clearly important to examine this question as closely as possible.

Restriction endonuclease cleavage sites were first mapped on a pox virus genome by Wittek *et al.*⁵ and this technique has been used by Mackett and L.C.A.⁶ to study the structure and relatedness of the genomes of several orthopoxviruses. Here we report the early results of an investigation of monkeypox variants, both by phenotype and by DNA analysis, to assess whether any such variants can be considered identical with whitepox viruses in all ascertainable respects.

Cloning technique

Stage 1: Material sampled from a single pock on the CAM was homogenized by manual shaking with sterile glass beads and 1 ml of 4 mM McIlvaine buffer, pH 7.4. The extract was then treated in an ultrasonic bath (Megasonic, 80 kc) for 1 min and

centrifuged at 1,000g for 5 min. The supernatant was filtered through a Millipore filter, average pore size 650 μ m, to remove most of the aggregates of virions. Filtration reduced the titre by about 90% and was assumed to leave a predominantly monodisperse suspension; suspensions were too dilute to check this by electron microscopy.

Stage 2: Dilutions were made immediately of the filtrate from stage 1 and were inoculated on the CAM and incubated for 64 h at 35 °C. A piece of membrane bearing a single pock was excised from a membrane having no other visible pock lesion, extracted as described above, and the extract inoculated on the CAM to produce stock virus. It was considered that two sequential 'clonings' resulted in a population which could be reasonably considered as the progeny of a single virion. Note that a 'clone' need not have invariant properties but might itself become genetically heterogeneous during multiple cycles of replication. Stability of biological properties was not taken as a criterion for the purity of a clone, although such constancy was found in practice for most of the clones studied.

Phenotypic characterization

Four markers were used in these experiments, each of which differentiated variola or whitepox from monkeypox. (1) Pock type. Monkeypox virus produces small, pink, haemorrhagic (h⁺) pocks on the CAM at 35 °C. Variola or 'whitepox' viruses give small, non-ulcerated white (h) lesions. The 'white pock' character was used for the primary selection of the variants, so that the class of monkeypox variants described here all produce white pocks on the CAM, but are not necessarily whitepox viruses as defined above. (2) Ceiling temperature. Monkeypox virus, but not variola virus, will produce pocks on the CAM of eggs incubated at 39 °C (ref. 7). In the experiments described here ceiling temperature was tested in the CV-1 line of monkey kidney cells which supported the growth of all relevant viruses at 35 °C, but at 40 °C monkeypox virus (t⁺) would grow and variola virus (t) would not. Note that embryonated eggs, being exothermic, maintain a temperature above the recorded temperature of the incubator⁸ and that ceiling temperatures determined in cell cultures may therefore seem to be up to 1 °C higher than those measured on the CAM. (3) Haemagglutinin. Most strains of variola do not produce detectable haemagglutinin (HA) for

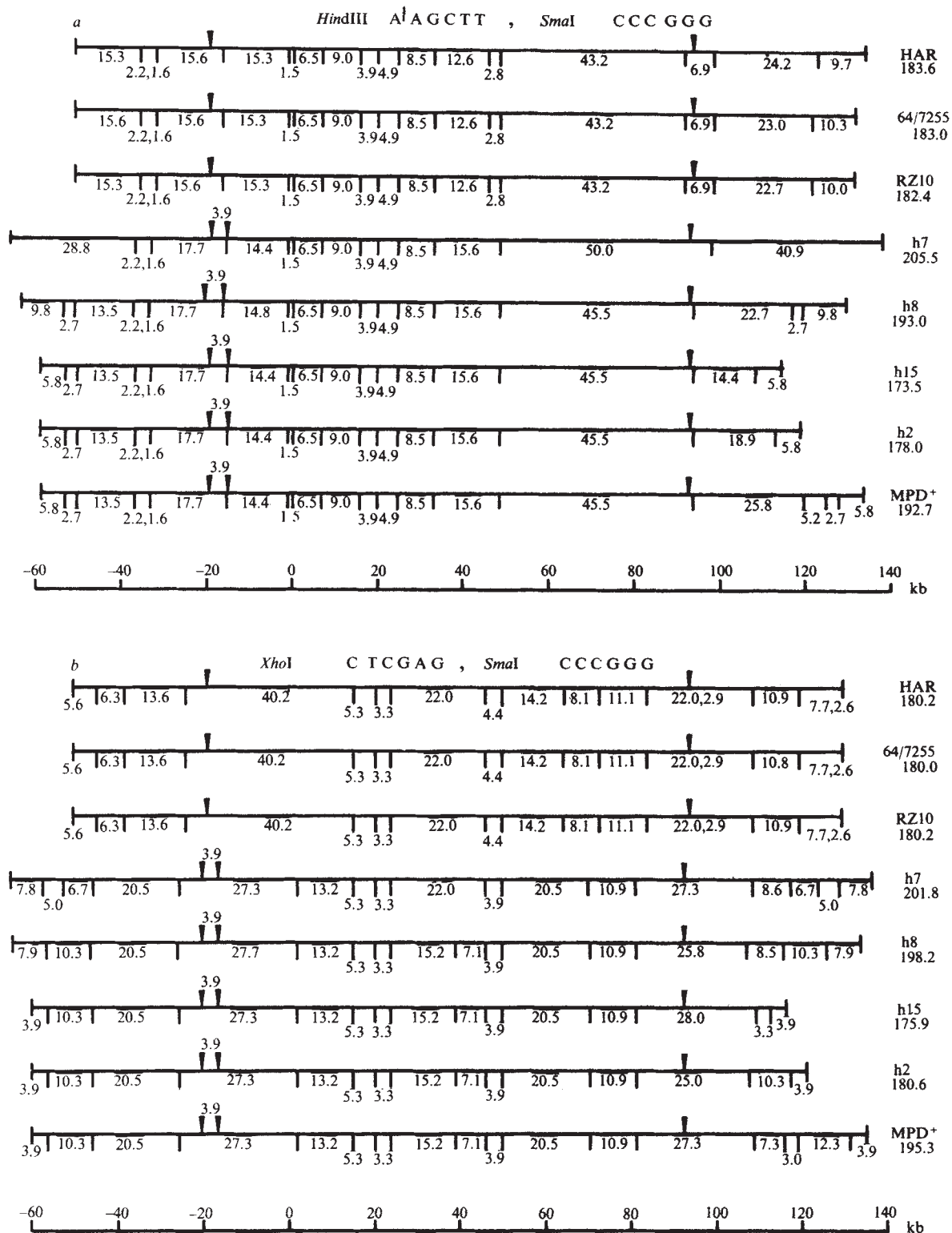


Fig. 1 Physical map locations of *Hind*III or *Sma*I restriction fragments (a) and *Xho*I or *Sma*I restriction fragments (b) of DNA from: variola Harvey (HAR), whitepox viruses 64/7255 and RZ10, monkeypox Copenhagen (MPD) and white pock mutants of MPD (h2, h7, h8, h15). Numbers indicate estimated sizes of individual fragments in kilobases (kb).

vaccinia-sensitive fowl erythrocytes when inoculated into RK13 cell cultures; monkeypox, on the other hand, makes large amounts of HA in RK13 cells⁹ and some is produced by two of the whitepox viruses¹⁰. In our study, viruses were classed as HA producers in RK cells (rh⁺) if 8 or more units were produced in standard HA titration¹⁰. (4) Growth in PEK cells. Marennikova *et al.*¹¹ reported that variola and whitepox viruses could be

passed readily in a pig embryo kidney (PEK) cell line, but that monkeypox viruses were lost on passage. Ueda and K.R.D. (unpublished) confirmed that variola and vaccinia replicated to high titre in a line of pig kidney cells. Strains of monkeypox virus either did not replicate or reached only low titres in these cells and were classed as 'p'. Whitepox viruses all replicated in these cells to significant titre (p⁺).

Isolation and phenotype of the h mutants

When the cloned wild-type monkeypox strain Copenhagen was inoculated on the CAM at approximately 50 pock forming units (PkFU) per membrane, less than 0.1% of the pocks were white. Two clones (h2, h15) were isolated.

The frequency of white pocks was significantly increased after passage of the wild stock on the CAM in high concentration. After four passes with an inoculum of $10^{6.0}$ PkFU, white pocks were present as 2–3% of the total and after six such passes, 50–60% of pocks were white. In a control series of eggs inoculated with 10^3 PkFU the frequency of white pocks remained at 0.2% after four and six passes. One clone (h3) was isolated after four passes at high concentration and seven (h4–h10) after the sixth such passage.

In subsequent experiments, two clones (h5 and h7) were re-passaged four times in high concentration and then recloned to give h5/1, h5/2, h7/1 and h7/3. Clone h7 was also recloned several times successively and stocks were made from the fifth and seventh reclonings (h7/c5, h7/c7). Another mutant (h16) was derived from a culture inoculated with parental monkeypox virus and incubated for 48 h in arginine-free medium, followed by a further 24 h in complete medium.

The phenotypes of all the h mutants are shown in Table 1. It will be seen that three clones (h2, h15, h6) are like the wild type except for the white pock character used in their selection. Five clones (h3, h4, h8, h9, h10) have one phenotypic change other than the white pock and two clones (h5, h7) resemble whitepox in three of the four markers used. Clones h5 and h7 did not change phenotype following passage in high concentration and recloning (clones h5/1, h5/2, h7/1, h7/3). None of the clones in this series had a lowered ceiling temperature, and this distinguishes them all from whitepox virus. However, a white pock mutant with a low ceiling temperature has been isolated from another strain of monkeypox and by using a different selection procedure. It is probable, therefore, that in an extended series, mutants would be found which were phenotypically like whitepox in all four characters used.

DNA analysis

Methods for virus growth and purification, extraction and cleavage of DNA and characterization of DNA restriction fragments were essentially as described previously^{6,12}.

Physical maps of cleavage sites were constructed mainly by hybridizations between restriction fragments generated from genomes of closely related viruses on the basis that, with the exception of terminal repetitions, fragments having sequence homology represent geographically equivalent regions of the genomes. Maps were also constructed by hybridizations between fragments cleaved from the same virus DNA by different restriction endonucleases. In some cases, physical maps of DNA from virus variants may be constructed from unequivocal interpretation of changes in the size of fragments generated by endonucleases *Hind*III, *Xho*I or *Sma*I and various double digests compared with those from wild-type DNA.

Comparative descriptions of the genomes of different orthopoxviruses and of changes between wild type and mutants are confusing when DNA fragments are labelled separately in order of size for each virus and for each restriction endonuclease. Additionally, it is impractical to measure from one end of the genome as most variation between and within orthopoxvirus types is found near the termini⁶ and, even within a single strain of virus, variations are found in the length of the terminal fragments between one cloned line and another¹³. It was decided to select one arbitrary reference point in an internal region of the genome; we chose the site separating the contiguous *Hind*III fragments O and I in the original map of rabbitpox⁵. Fragments of similar size map in equivalent locations in all orthopoxvirus genomes for which *Hind*III maps have been constructed⁶, and this region of the genomes is highly conserved. The reference point separates wild-type monkeypox *Hind*III fragments Q and I⁶. Locations of cleavage sites for particular

endonucleases are defined as distances from the reference point measured in kilobases, with a positive sign if to the right and a negative sign if to the left. Sizes of genomes were derived by summation of restriction fragments sized by co-electrophoresis with digests of wild-type DNA. Physical maps were drawn to scale and the sizes of individual fragments indicated in kilobases (Fig. 1).

Physical maps of the cleavage sites for endonucleases *Hind*III, *Xho*I and *Sma*I in the genomes of two strains of variola and of three strains of wild-type monkeypox including Copenhagen (MPD) have been presented previously⁶. Physical maps for the same three endonucleases have been constructed from hybridization data and confirmed by double digestion for two of the whitepox viruses (64/7255 and RZ10) and are shown in Fig. 1. These are the first maps of whitepox viruses to be constructed, although electropherograms of endonuclease digests of whitepox DNA have been presented previously¹⁴. Viruses 64/7255 and RZ10 map very similarly to variola strain Harvey (HAR), preserving all characteristic internal cleavage sites but showing small variations in the size of the *Hind*III termini and of near-terminal *Hind*III or *Xho*I fragments mapping between 100 and 120 kilobases. The sizes of *Hind*III, *Xho*I or *Sma*I restriction fragments from the genomes of the other four whitepox viruses and of two whitepox-like viruses (Ham 8, Ham 9) isolated from hamsters³ are similar to those presented here.

A general feature of orthopoxvirus genome structure is the presence of species-specific, terminal repetitions containing subsets of common sequences^{6,15}. Because of this, the opposing terminal fragments from any particular genome cross-hybridize and either terminus will hybridize to both termini of the genome of another orthopoxvirus. A structural feature peculiar to variola and whitepox viruses is that the opposing termini fail to cross-hybridize. Additionally, the MPD/*Hind*III 2 molar terminal 5.8-kilobase fragment hybridizes specifically with only right-hand terminal fragments and the 2 molar MPD/*Hind*III 2.7-kilobase fragment with only left-hand terminal fragments of variola-like genomes. Such genome organization in relation to that of other orthopoxviruses may have resulted from non-symmetrical deletion of sequences within hypothetical repetitions or, from exchange of specific repetitive sequences between termini suggesting conjunction of the termini at some stage of replication.

Three sorts of genome have been obtained from the different white pock mutants of monkeypox (MPD). One (h16) has no change detectable by analysis with *Hind*III, *Xho*I or *Sma*I. White pock mutants of other monkeypox strains having a similar relationship to the respective parents have also been isolated. Second, the two spontaneous MPD white mutants (h2, h15) map with large deletions close to the right-hand terminus and another, not fully characterized, seems to be of the same class. The deleted sequences overlap partially and represent about 15 and 19 kilobases respectively. Variation at this locus is common in orthopoxvirus genome structure⁶. Third, white pock mutants isolated following high multiplicity passage (h3–h8, h10) show

Table 1 Phenotype of monkeypox virus and its 'h' mutants

Phenotype		Viruses
h ⁺ t ⁺ rh ⁺ p		Monkeypox, wild type
t ⁺ rh ⁺ p	h	h2, h6, h15
t ⁺ rh ⁺	h p ⁺	h3, h4, h8, h9
t ⁺ p	h rh	h10, h16
t ⁺	h rh p ⁺	h5, h7
t ⁺	h rh p ⁺	h5/1, h5/2, h7/1, h7/3, h7/c5, h7/c7
rh ⁺	h t p ⁺	64/7255, 64/7275 (whitepox viruses)
	h t rh p ⁺	Chimp 9, Mk7, RZ10, RZ38, (whitepox viruses)

Pocks: haemorrhagic, h⁺; white, h. Growth in CV1 at 40 °C, t⁺; no growth, t. Haemagglutinin in RK13 cells: titre ≥ 16 , rh⁺; <8 , rh. Growth in PEK cells; good, p⁺; none, p.

major and complex changes involving both termini of the genome symmetrical and smaller changes at some internal sites. Mutants h3-h7 and h10 are essentially similar and have genomes about 7-9 kilobases larger than that of the wild type. Assuming that symmetrical, near-terminal fragments generated by endonuclease *XhoI* result solely from cleavages within the terminal repetitive sequences, the repetition is then both larger than and divergent in sequence from that of the wild-type genome. A minimum estimate of the size of the parental repetition by summation of the symmetrical near-terminal fragments is 8.5 kilobases, whereas that for white mutants of this type is 19.5 kilobases and contains no sites for endonuclease *HindIII*. With the exception of h3, the genomes of these mutants have also lost a *XhoI* site in an internal, normally highly conserved region at the 38-kilobase position. A *XhoI* site at this locus, present in the genome of mutant h6 when isolated, was lost on subsequent recloning. Mutant h8 also shows extensive, symmetrical, near-terminal sequence rearrangements; the minimum size of the terminal repetition is 18 kilobases and the *HindIII* sites defining the MPD 2.7 kilobase fragment are retained. Mutant h8 is similar to h2 in some respects, showing a near-terminal deletion (3-4 kilobases) mapping to the right of the *SmaI* site located at 92 kilobases, and symmetrical, near-terminal sites for *XhoI*. Additionally, h8 has an apparent insertion, relative to the parental genome, of 0.4 kilobases mapping between -1.5 kilobases and the *SmaI* site at -15.5 kilobases. A similar small insertion is seen in the same region of the genome of mutant h5. Mutant h8 retains the internal *XhoI* site at the 38-kilobase position. The locations of *SmaI* sites, relative to those in wild-type DNA, are preserved in all the monkeypox mutants described.

The rearranged genome exemplified by mutant h7 is apparently a stable configuration as neither four further high multiplicity passages followed by recloning nor nine successive reclonings generated further changes detectable by restriction analysis with *HindIII*, *XhoI* or *SmaI*.

Although the genotypes of the spontaneous mutants h2 and h15 are readily recognizable as variants of wild-type monkeypox, the changes exhibited by, for example, mutants h7 and h8, seem to be as extensive as those distinguishing some orthopox-virus species⁶. However, the genomes of these mutants remain readily distinguishable from those of variola and whitepox viruses.

Discussion

Monkeypox mutants. The objective of the present study was to characterize mutants of monkeypox virus, both phenotypically and by DNA analysis, and to assess how closely they might mimic whitepox or variola. The mutants were derived in different experimental conditions but each was selected initially from a white pock produced on the CAM.

Spontaneous white-pock mutants are relatively rare in monkeypox. The two which were obtained each had a genome substantially shorter than that of MPD due to deletion of DNA sequences near the right-hand terminus, together with some rearrangement of terminal sequences. The mutants obtained after high-multiplicity passage had more complex, symmetrical rearrangements of terminal sequences and the genomes were as big as or bigger than that of MPD. Smaller deletions of sequences were detected in some of them in approximately the same locus as the large deletions in the genomes of the spontaneous mutants. Although the numbers are small, it seems that particular experimental conditions affect the kind of mutant

most likely to be selected. It is suggested that the spontaneous mutants may represent an early, and the high-multiplicity mutants a late stage in a process which can engender complex changes in genome structure during replication. This hypothesis is now being tested. Several phenotypically different mutants had genomes with maps similar to those shown for mutant h7. The genome of h7 seems to represent a stable configuration. The maps of h7 showed no detectable changes after further passages at high multiplicity and several reclonings. The symmetrical nature of the changes which occur at the two ends of the genome strongly suggest that the near-terminal configurations had been determined in the same process or that sequences from one end had been copied to the other. On either basis, the evidence supports the previously proposed^{16,17} idea of transient circularization at some stage in the replication of orthopoxvirus DNA.

The mutants exhibited a variety of phenotypic changes but it was not expected that these could be correlated with the changes in the relatively crude restriction maps; indeed, one mutant (h16), with no changes detectable in the maps, had two changes in the phenotypic markers.

Whitepox and monkeypox. No phenotypic character has yet been described which distinguishes the group of whitepox viruses from variola. Consistent with this, the maps of the genomes of the whitepox viruses are closely similar to those of variola HAR, and they share with variola the failure of one terminus to cross-hybridize with the opposing terminal fragment. Although the present maps are too crude to prove identity, they add to the steadily growing evidence of the close relationship of whitepox to variola.

On the other hand, there are significant differences between the genomes of whitepox and monkeypox. Apart from the differences in near-terminal sequences, there are four cleavage-site differences within the conserved region of the genome. The *SmaI* site at -15 kilobases and the *XhoI* sites at 3 and 38 kilobases are present in monkeypox but not in variola or whitepox, whereas a *HindIII* site at 45 kilobases is present in variola and whitepox but not in monkeypox. Of these four sites, the only one found to be changed in some of the h mutants is the *XhoI* site at 38 kilobases. Even the two mutants (h5 and h7) which resemble whitepox in three of the four phenotypic markers have genomes which differ significantly from that of variola. On the other hand, there is evidence that different experimental conditions select for different kinds of complex mutations and some caution is necessary in forecasting what limits could be set to the possible variations in the monkeypox genome.

Our experiments have failed to generate any monkeypox mutants with genomes similar to those of the whitepox-like viruses described by Marennikova and Shelukhina³; however, the methods used in the generation of our mutants differ from those described by these authors.

A crucial question is whether variola virus possesses any DNA sequences unique to itself and which are missing from monkeypox (and even perhaps from whitepox), as rearrangements, however complex, could not be expected to create significant new information. This question cannot yet be answered, but several ways of approaching it are theoretically possible and one main reason for any continuing work with variola would be to obtain the answer.

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Structure of southern bean mosaic virus at 2.8 Å resolution

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X-ray diffraction studies reveal that the polypeptide chain of the southern bean mosaic virus protein subunit has a fold closely similar to the shell domain of tomato bushy stunt virus. The protruding domain of tomato bushy stunt virus is absent in southern bean mosaic virus. The tertiary structure observed in these viruses may be particularly suitable for the formation of the protein coat in small, spherical, RNA-containing, plant viruses.

SIMPLE plant viruses are composed of nucleic acid and a protein coat consisting of multiple copies of covalently identical subunits arranged in a symmetrical manner^{1,2}. These viruses can be broadly categorized as rod-shaped, bacillus-shaped and spherical. The morphological classes can be subdivided into groups based on physical and biological properties such as stability, symmetry and strategy of replication^{3,4}. High resolution X-ray structure determinations have been accomplished for the rod-shaped tobacco mosaic virus⁵ (TMV) and its disk protein⁶ as well as the spherical tomato bushy stunt virus⁷ (TBSV). The contrasting structures of the proteins in these two viruses reveal differing requirements for assembly into a helical rod and icosahedral shell. Although TMV protein subunits consist primarily of four, roughly radial, α -helices, the two domains of TBSV are composed almost entirely of anti-parallel β -pleated sheet. A possible common feature in the two virus classes is the flexibility of the protein which interacts with the RNA. Although TMV is the only rod-shaped virus being studied at high resolution, several spherical plant viruses, representing different groups, are being investigated (ref. 8, and P. Argos and P. Colman, personal communications). We report here the structure determination of southern bean mosaic virus (SBMV) seen at 2.8 Å resolution. Although SBMV represents a group of viruses⁹ with properties significantly different from those of the TBSV group, its viral envelope was found to possess a tertiary and quaternary structure closely similar to that of the TBSV shell (S) domains. It is proposed here and also by Argos¹⁰ that other groups of small, spherical, RNA-containing, plant viruses may also be based on the same folding and assembly principles.

Structure determination

The high resolution structure of SBMV was determined using the rhombohedral type II SBMV crystals¹¹ with hexagonal axes of $a = 334.3$, $c = 757.5$ Å and a single particle in the unit cell. The R32 space group requires the particle to be situated on a site with 32 crystallographic symmetry, thus placing 10 icosahedral units within the crystallographic asymmetric unit (Fig. 1a).

Data for the native crystals and four heavy atom derivatives were collected using oscillation photography¹²⁻¹⁵. The X-ray source was an Elliott rotating anode generator, equipped with a 0.1-mm focal cup and a copper target. The Ni-filtered radiation was focused with two perpendicular mirrors¹⁶. Some details of the data collection are shown in Table 1a. In total, 2,022,187

useful intensities were measured which permitted the determination of phases for 275,966 independent reflections with the multiple isomorphous replacement method. This represented 71% of all possible reflections within the limiting 2.8-Å resolution sphere.

The analysis of the first heavy atom derivative was given previously¹⁷ as were the results of a 5-Å resolution electron density map¹⁸. The heavy atom positions of four derivatives were found with difference electron density maps and refined by a least-squares procedure¹⁹. The final atomic parameters will be given elsewhere²⁰, but some statistics for the multiple isomorphous replacement phase determination are shown in Table 1b.

The final 'best' multiple isomorphous replacement (MIR) electron density map was averaged over the 10 different icosahedral units within one crystallographic asymmetric unit²¹. This map was originally used to solve the SBMV coat protein structure. One cycle of phase refinement using the molecular replacement method²²⁻²⁴ has been completed which provided some improvement in the apparent resolution. For convenience and in accordance with general practice these maps will be referred to as having 2.8 Å resolution, even though the phasing and quality of data are less at high resolution than at low resolution. A more full account of the crystallographic work is reported elsewhere²⁰.

The averaged MIR electron density map was displayed in sections perpendicular to an icosahedral 2-fold axis. The quasi-3-fold axis relating the three subunits within the icosahedral unit was immediately obvious (Fig. 1b). The effect was particularly striking because this axis was nearly parallel to its neighbouring icosahedral 2-fold axis and, hence, perpendicular to the map sections. A similar situation pertains to TBSV²⁵. The dominant feature in the SBMV map was a ' β -barrel' type structure (see ref. 26) in which the extended strands were well resolved from each other. A substantial portion of the polypeptide chain could be traced but some ambiguities remained in the connectivity of the strands at the end of the β -sheets. Five helices could be recognized and these determined the direction of the polypeptide. Certain features, in particular the amino-terminal arm, present in only the C subunit (Fig. 1c), were then seen to be similar to the TBSV structure. Superposition of the TBSV C_{α} co-ordinates onto the SBMV electron density map revealed a remarkable resemblance of the TBSV shell domain and the SBMV subunit. The TBSV co-ordinates had been referred to an icosahedral axial system and were used without change in radius on the SBMV map. This permitted an interpretation of all turns and bends on the original MIR map which was subsequently readily verified on the molecular replacement map. These results demonstrated the similarity not only of fold but also of the

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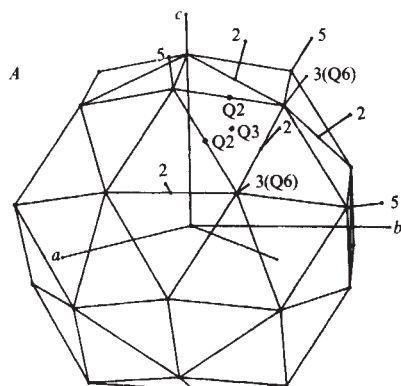


Fig. 1 *A*, The complete viral shell composed of 60 triangular icosahedral asymmetric units showing the quasi-symmetry axes Q2, Q3 and Q6. The quasi-3-fold axis Q3, for instance, relates three covalently identical but conformationally slightly different A, B and C subunits to form 1/60th of the icosahedral shell. *B*, View through three sections of one triangular unit as defined in *A* ($z = 126-129$ Å) to show the quality of the averaged molecular replacement map after one iteration. Note the quasi-3-fold relationship between the three subunits in the icosahedral asymmetric unit. The pentagon and hexagon symbols represent icosahedral 5-fold and quasi-6-fold axes, respectively. Contours are chosen at equal intervals of 1/10th of the maximum protein height. Only those contours above the mean map value are shown. The lowest contour is represented by a dashed line. *C*, Arrangement of subunits A, B and C within one icosahedral asymmetric unit as viewed from the inside of the virus, looking outward. This view permits a representation of the amino ends of the C subunits.

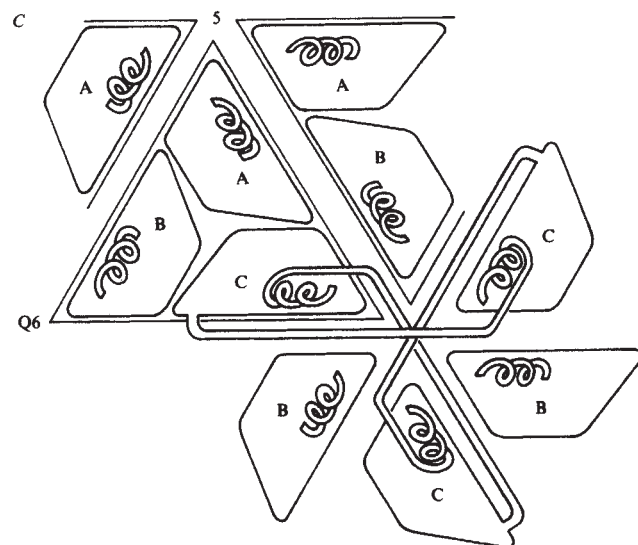
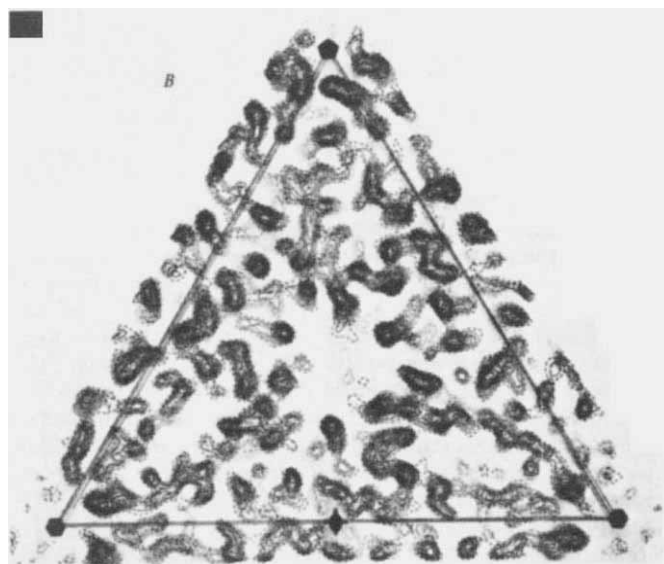


Table 1 Some data collection statistics (*a*) and multiple isomorphous replacement refinement parameters (*b*)

Compound	Resolution limit (Å)	Oscillation range	No. of reflections measured	No. of independent reflections	<i>R</i> (%)	Rejection criterion			
<i>a</i>									
Native	2.8	0.5°	652,887	321,586	13.5	None			
	3.5	1.0°	346,412	175,788	13.1	None			
PHMBS	2.8 combined		438,759	299,893	12.5	<i>F</i> ² < 1σ			
	2.8	0.5°	633,954	318,227	15.6	None			
	3.5	1.0°	357,937	178,606	12.2	None			
	2.8 combined		430,717	289,234	11.3	<i>F</i> ² < 1σ			
K ₃ (UO ₂)F ₅	2.8	0.5°	631,979	320,184	16.5	None			
	4.0	1.25°	188,711	100,739	14.5	None			
	2.8 combined		358,606	280,857	14.4	<i>F</i> ² < 1σ			
	3.5	1.0°	309,233	157,065	11.1	<i>F</i> ² < 1σ			
AuCl ₄	3.5	1.0°	278,156	149,992	12.2	<i>F</i> ² < 1σ			
<i>b</i>									
Shell Resolution (Å) ∞	1	2	3	4	5	6	7	8	Overall
	22.2	11.1	7.4	5.6	4.4	3.7	3.2	2.8	
Figures of merit:									
< <i>m</i> >	0.70	0.71	0.75	0.72	0.65	0.61	0.53	0.46	0.59
<i>n</i>	430	6228	15,963	30,853	42,879	64,440	69,530	45,643	275,966
<i>R</i> -factors									
PHMBS	31	28	25	33	33	37	44	61	42
K ₃ (UO ₂)F ₅	35	36	31	46	40	51	75	96	55
K ₂ PtCl ₄	115	76	75	59	53	50	40	—	45
AuCl ₄	41	50	58	49	52	47	52	—	53

PHMBS, *p*-hydroxymercuribenzoate sulphonate. $\langle m \rangle$ is the mean figure of merit. n is the number of phased reflections in shell.

$$*R = \frac{\sum_h \sum_i |F_{hi}^2 - F_h^2|}{\sum_h \sum_i F_h^2} \times 100$$

where F_h^2 is the mean of the i observations F_{hi}^2 on the reflection h .

$$R = \frac{\sum [|F_{HV}| - (|F_V| + f_H)]}{\sum |f_H|} \times 100$$

where F_{HV} , F_V and f_H are the structure factors for the heavy atom derivative, the native virus crystals and the heavy atoms alone, respectively.

packing of protein subunits in the viral shells. Backbone models of the C subunits (see Fig. 1b) were built into a Richards optical comparator²⁷ with Kendrew-Watson model parts on a scale of 2 cm = 1 Å. The co-ordinates of the SBMV backbone atoms were measured with a plumb line and checked for gross error. These have been deposited with the Protein Data Bank at Brookhaven National Laboratory²⁸.

The chain tracings of the C subunit, together with the corresponding structures of the quasi-3-fold-related A and B subunits, accounted for all the major features in the map. The internal boundary of the protein shell was well defined. Features of the electron density in the interior of the virus were lower and disconnected in the averaged maps, permitting no explicit recognition of nucleotides.

The 2.8-Å resolution map did not differ in substance from the earlier 5.0-Å map¹⁸; indeed, the electron density distributions were remarkably similar. The selection of the subunit boundaries had been essentially correct in the low resolution map. The feature referred to as a 'small lobe of the subunit' was now seen to be the extra protruding helix near the quasi-3-fold axis in SBMV (*vide infra*). The 'large helix-containing lobe' did not, in fact, contain helix but was related to the very obvious protrusions near the 5- and quasi-6-fold axes. The sheet near the 2-fold axes corresponded to one side of the β -barrel. The '26 distinct phosphate sites' seen at 5 Å resolution must now be regarded with some scepticism. Although these features within the RNA region remain in position, they are lower in density relative to the protein in the averaged isomorphous replacement map.

The protein shell and viral exterior

The SBMV subunit is primarily an eight-stranded anti-parallel β -barrel (Fig. 2). However, at least 66 residues at the amino end are not a part of the barrel, but form a partially ordered 'arm' in the interior of the virus. The protein shell of SBMV is about 35 Å thick, extending from around 110 Å to 145 Å radius. Each quasi-3-fold axis is 27 Å distant and parallel to its nearest icosahedral 2-fold axis. A careful analysis of the conformational differences between the three quasi-symmetrically related subunits A, B and C has not been completed. An initial examination of the electron density and position of equivalent heavy atoms²⁰ shows that the A subunits differ significantly from the B and C subunits at turns close to the 5-fold axes, where SBMV has insertions relative to TBSV. Other regions show less deviation, as was also the case in TBSV⁷, where differences were less than 1 Å.

Figure 3 shows a superposition of the C $_{\alpha}$ coordinates for the S domain of TBSV and the SBMV subunits. Both structures are referred to the same icosahedral axial system. The r.m.s. difference in C $_{\alpha}$ coordinates is 2.7 Å over 182 residues, excluding those residues which are insertions or deletions in one or the other subunits. The extent of topological equivalence is almost as good as that between the α and β chains of horse haemoglobin and is certainly much better than that between the NAD-binding domains of lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase (Table 2).

Harrison (personal communication) has assigned a tentative amino acid numbering system to the TBSV C subunit which is shown in Figs 2 and 3, and shall also be used in SBMV. Some residues may have to be inserted or deleted pending the full amino acid sequence determination of TBSV and SBMV. The ordered amino-terminal arm of the TBSV C subunit runs from 1 to 32 followed by the S domain from 33 to 200. The TBSV P domain consists of the final 111 residues within the polypeptide chain. Major differences between the SBMV subunit and the TBSV S domain are (1) an extra α -helix (see following section), a feature which is ordered in all three quasi-equivalent subunits, (2) the organization of the β -annulus structure about the icosahedral 3-fold axes, (3) insertions of residues 49A and 177A, 177B, 177C, 177D near the quasi-6-fold and icosahedral 5-fold axes, (4) insertion of 17 residues between 155 and 160, replacing residues 156–159 near the quasi-3-fold axis, (5) deletion of

residues 55–61 opposite the TBSV P domain on the viral exterior and replacement by residue 55A, (6) deletion of residue 104 at a subunit interface, (7) deletion of residue 114 and insertion of residue 118A at a different subunit interface near the viral surface, and (8) deletion of residue 130 and insertion of some residues at the protein-RNA interface.

The most dramatic alterations between TBSV and SBMV occur on the viral surface. The larger protruding P domains about the 2-fold axes in TBSV are replaced by the abbreviated protrusions around the quasi-3-fold axes in SBMV (Fig. 4a). The SBMV protrusions are formed by the insertion of a three-turn helix (155A to 155M) running tangential to the viral surface and a one-turn addition (155N–155Q) to the radial helix 160–164. The latter makes a 60° left-handed turn over a distance of 12 Å about the quasi-3-fold axis, thus forming a keratin like structure²⁹ when taken in conjunction with its two neighbouring subunits. There is also a protrusion around the icosahedral 5-fold (Fig. 4b) and quasi-6-fold (Fig. 4c) axes in SBMV which is absent in TBSV. Both the trimer (Fig. 4a) and the pentamer (Fig. 4b) – hexamer (Fig. 4c) clustering in SBMV had been observed previously in a 22.5-Å resolution map³⁰. The third major difference is the deletion of TBSV residues 55–61. These residues make an excursion, towards the TBSV P domain, out of the β -strand that runs between residues 52–62. They stabilize the orientation of the P domain in the C subunit, where the hinge between the domains is bent more acutely than in the A and B subunits. This function is not required in SBMV. The evolution of surface differences between TBSV and SBMV might be a recognition mechanism for altering host specificity by adapting to the properties of host or vector.

Both TBSV and SBMV require metal ions for assembly and stabilization of their protein shell. The function of calcium and magnesium ions has been extensively studied with respect to the properties of SBMV^{31–34}. In the absence of metal ions and at alkaline pH these viruses swell by about 7% in radius. Wells and Sisler³⁵ have shown that swollen SBMV has only 2% of the infectivity of native SBMV. This could be due to the loss of integrity of the viral surface features or it might be the consequence of the ease with which the swollen virus can be degraded by nucleases and proteases. The positions of the metal ions in TBSV have been determined by Harrison *et al.* (ref. 7 and personal communication). There are significant conformational differences (Fig. 3) between TBSV and SBMV in the region (residues 113–120) of this site.

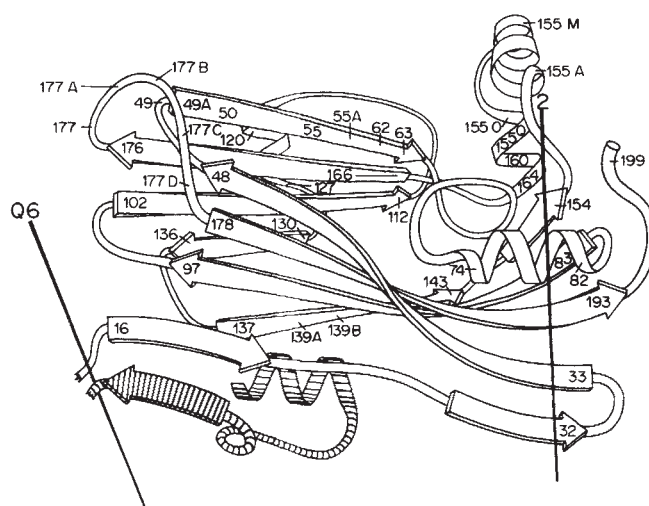


Fig. 2 Diagrammatic representation of the SBMV C subunit with its amino-terminal arm and α -helix. The latter is bound to the base of a subunit, possibly related by a 3-fold rotation. The very tentative amino acid numbering scheme is shown. Numbers such as 177A, 177B are insertions in SBMV relative to TBSV. Drawing by Jane Richardson.

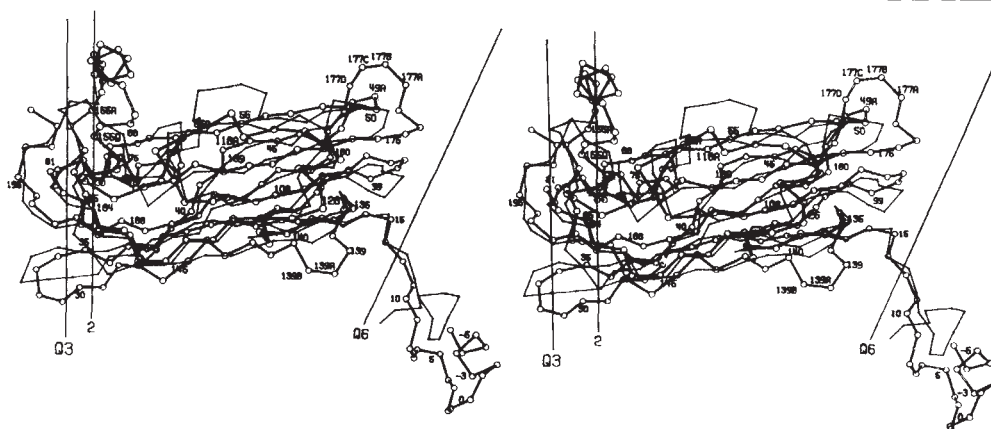


Fig. 3 Stereo diagram showing the C_{α} atoms in SBMV (large circles connected with thick bonds) superimposed on the TBSV shell domain (thin bonds). Both viruses are referred to the same icosahedral axial system. The amino acid numbering of SBMV is shown. It relates to a tentative system for TBSV

The amino acid composition of SBMV has been determined by Tremaine³⁶, Ghabrial *et al.*³⁷ and Dietrich³⁸. Dietrich has also partially determined the amino acid sequence of 15 tryptic and 2 cyanogen bromide fragments. Attempts to find the position of these peptides (the largest being 16 residues long) in the electron density map were only partially and tentatively successful.

The RNA-protein interface

The structure of SBMV as well as TBSV has the interesting feature of an 'amino-terminal arm' which is ordered only in the C subunits. It is positioned between quasi-6-fold-related C and B subunits and runs from a quasi-6-fold to an icosahedral 2-fold axis. Harrison *et al.*⁷ point out that the space for this arm can only exist in the vicinity of an icosahedral 2-fold axis (the quasi-3-fold axes are parallel to the icosahedral 2-fold axes but make an acute angle with the quasi-2-fold axes). The differences of the arm structure are required to accommodate the quasi-symmetry of the $T=3$ surface arrangement. The disordered arm in the A and B subunits must thus be closely associated with the RNA but in variable positions relative to the icosahedral coat. Chauvin *et al.*³⁹ have shown with neutron scattering of suitably matched solutions of TBSV with D_2O that the significant portion of this protein-RNA interaction is at a radius of less than 80 Å.

In the SBMV protein there is a structural feature situated between the base of the β -barrel and the RNA interior, consisting of an α -helix and extended polypeptide chain, that was not visible in the TBSV structure. This helix, interacting with the C subunit barrel, represents either the amino end of a neighbouring, 3-fold-related, C subunit (Fig. 2) or an insertion within the C subunit at position 139. The β -annulus structure around the quasi-6-fold axis in SBMV is a '2-way overlap', not a '3-way overlap' as in TBSV (Fig. 5a). The extra 'amino-terminal' helix and extended strand are clearly visible in the A, B and C subunits, but the β -annulus and amino-terminal arm (residues 8–32) are visible only in the C subunit (Fig. 5b). It is therefore possible that the extra 'amino-terminal' helix in subunits A

(forming the pentamer cluster) or in the B subunits (forming the hexamer cluster with the C subunits) are associated with their own β -barrel rather than a neighbouring subunit.

There are a total of 220 ordered residues in the SBMV C subunit. An error of only a few residues might be expected in view of the close similarity in the placement of amino acids in homologous regions of SBMV and TBSV. The total number of residues in SBMV has been estimated to be between 260 and 270 (refs 36–38). Thus, the ordered SBMV C subunit lacks between 40 and 50 residues. A similar estimate by Harrison *et al.*⁷ showed that there were probably around 50 disordered residues in the C subunit of TBSV, even without observing the extra 'amino-terminal' α -helix. The amino terminus of the extra α -helix in SBMV is buried by the β -annulus, leaving no room for any extension. It is therefore more likely that the missing polypeptide is inserted around residue 8, which has weak density and is accessible to the viral interior (Fig. 5b). Support for the occurrence and position of ordered and disordered protein segments comes from an analysis of heavy atom positions²⁰.

Sehgal has shown that swollen SBMV virions, produced by removal of calcium and magnesium, are susceptible to trypsin digestion⁴⁰, and Tremaine⁴¹ has shown that the cleaved portion is strongly basic and consists of about 63 residues. The treated virus reassembles into particles of diameter 150–180 Å. Similar observations have been made for brome mosaic virus (BMV, ref. 42 and B. Verduin, personal communication), TBSV and turnip crinkle virus^{43,44} and alfalfa mosaic virus⁴⁵ (AMV), with the cleaved fragment always being at the amino terminus. A β -annulus structure, near the amino terminus, and the insertion of the amino-terminal arm between subunits may thus be essential requirements for the formation of a hexamer cluster and hence in the construction of spherical viral coats with $T > 1$. If the amino-terminal segment is cleaved (including the β -annulus), only pentamer clusters can form, producing capsids similar to the satellite tobacco necrosis virus (STNV) with a diameter of around 190 Å.

The amino-terminal region of many spherical and bacillus-shaped plant virus coat proteins is often strongly basic (for example, SBMV, cowpea chlorotic mottle virus, BMV, AMV and STNV) and predicts as an α -helix where the amino acid sequence is known (see Argos¹⁰). In SBMV there are residues which form an α -helix binding to the base of the β -barrel at the protein-RNA interface. Thus, the side of the helix exposed to the RNA is likely to be basic and the other side hydrophobic. Indeed, the SBMV electron density shows that residue -3 (Fig. 3) is almost certainly arginine. These properties will make the amino-terminal region particularly susceptible to trypsin digestion once it has been sufficiently exposed⁴⁰.

There are on the average around 8 basic residues per subunit, where the amino-terminal arm sequence is known, providing a total of 1,440 positive charges within the RNA of the complete virus. As there are about 5,000 bases in the nucleic acid of SBMV, it follows that the amino termini together must contribute significantly towards the cancellation of charge within the virus and hence presumably are largely responsible for the RNA

Table 2 Quality of structural similarity between various proteins or protein domains

Comparison		Equivalent residues		% of equivalent residues	
Molecule 1	Molecule 2	No.	r.m.s. distance (Å)	Mol. 1	Mol. 2
TBSV(S)	SBMV	182	2.7	91.0	82.7
Hb(α)	Hb(β)	139	1.6	98.5	95.3
NAD domain	NAD domain				
LDH	GAPDH	83	2.9	57.5	56.1
TBSV(S)	TBSV(P)	69	3.8	41.3	62.8

Abbreviations: LDH, lactate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hb, haemoglobin. Equivalent residues is restricted to those in the same topological sequence³².

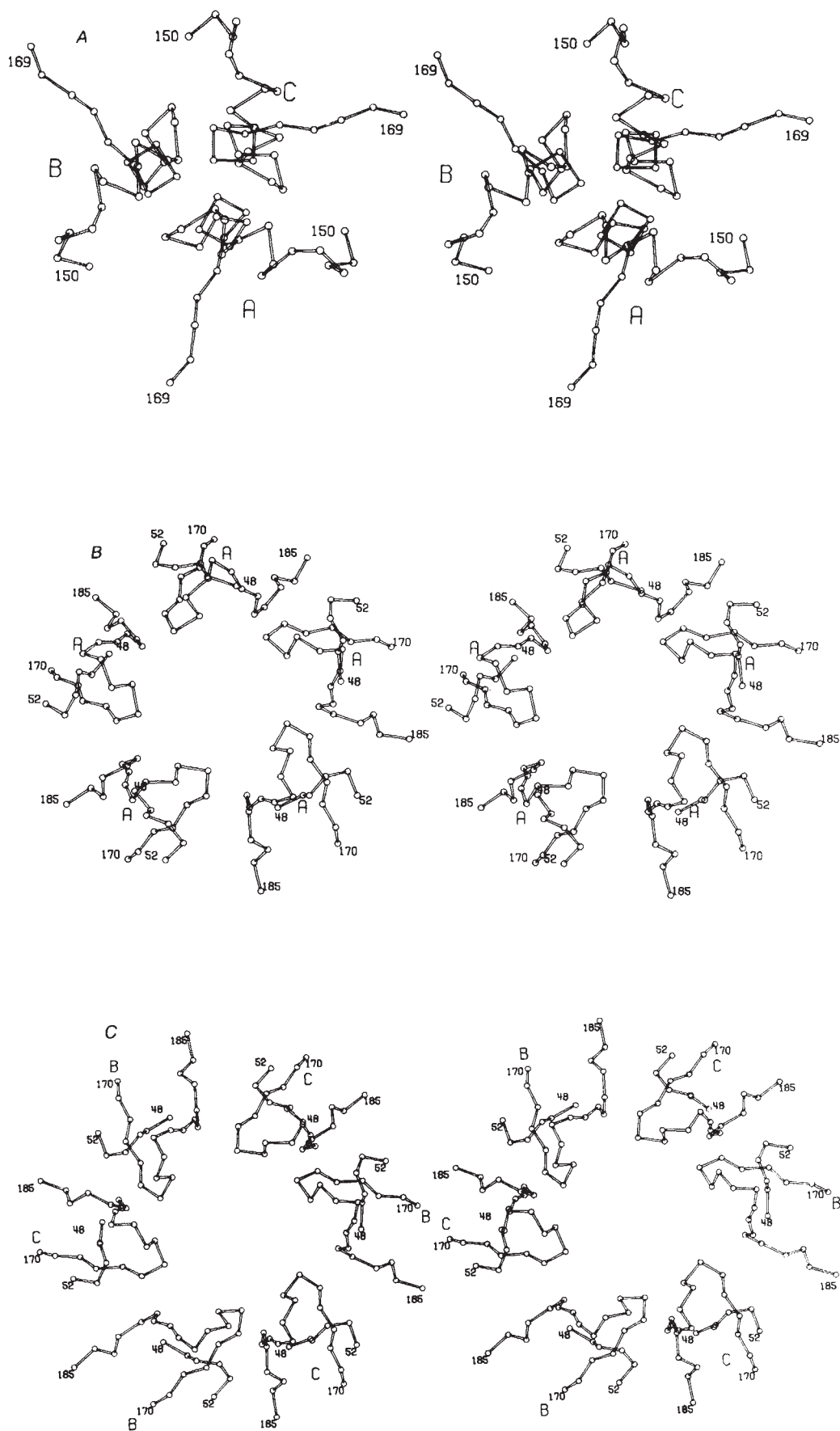


Fig. 4 Views of the viral exterior surface. **A**, Stereo diagram showing the protrusion around a quasi-3-fold axis produced by subunits A, B and C. The tangential α -helix occurs only in SBMV. The radial helix forms a keratin-like structure about the quasi-3-fold axis. **B**, Stereo diagram showing protrusion around an icosahedral 5-fold axis produced by adjacent A subunits. **C**, Stereo diagram showing protrusion around a quasi-6-fold axis produced by three C subunits separated by three B subunits.

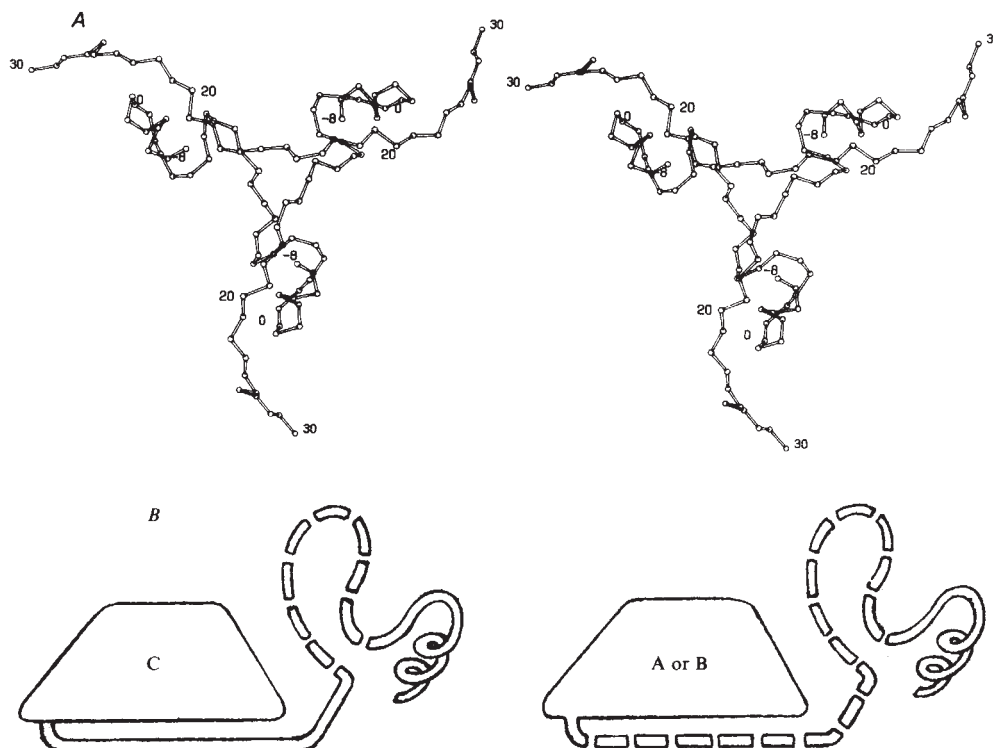


Fig. 5 A, Possible arrangement between C subunits in the vicinity of an icosahedral 3-fold axis which together form a 'β-annulus' structure. An alternative interpretation of the electron density places the amino-terminal helix as an insertion at position 139. B, Ordered and disordered (dashed) polypeptide in the A, B and C subunits of SBMV.

folding during viral assembly. The apparent tendency of α -helical formation with hydrophobic sides¹⁰ suggests that the RNA backbones will have closely associated α -helical protein which form hydrophobic 'bundles'. These in turn can pack more easily within the virus by exclusion of water. A similar situation is encountered in the formation of α -helices when protamines associate with tRNA and also probably with DNA⁴⁶.

Histones H2A, H2B, H3 and H4, like viral coat proteins, also have basic amino-terminal ends which are not part of the ordered nucleosome structure. The H3 and H4 histones apparently undergo an ordering phenomenon by binding to the linker DNA⁴⁷⁻⁴⁹. The ordering of protein incurred by the binding of RNA to TMV, or the requirement of the random amino-terminal segments to assemble spherical viral RNA, is analogous.

The structure of other small, spherical, RNA-containing plant viruses

The most surprising aspect in the present report is the observation that, in spite of the obvious differences in physical characteristics, the structure of SBMV is closely related to that of TBSV. Argos¹⁰ has shown that the amino acid sequence of some other viruses is consistent with a similar coat protein structure. Complementary arguments based on packing the SBMV shell protein are given here for the small $T = 1$ STNV particle. Indeed, it has already been observed that trypsin-treated SBMV^{40,41}, turnip crinkle virus^{43,44}, AMV⁴⁵ and BMV⁴² can form particles similar in diameter to STNV⁵⁰.

The overall dimensions of the SBMV subunit are given by a 35-Å thickness, a 27-Å separation between the quasi-3-fold and icosahedral 2-fold axes and a separation varying from 42 to 55 Å between the icosahedral 2-fold and quasi-6-fold axes. Similar measurements pertain to the thickness of the protein coat, the separation of the icosahedral 2-fold and 3-fold axes and the separation of the icosahedral 2-fold and 5-fold axes of the $T = 1$ STNV particle, respectively⁵⁰. Computer experiments in which pentamer clusters of SBMV A subunits are placed into a $T = 1$ icosahedral lattice confirmed that the subunits pack without overlap when the external radius is 88 Å along the 3-fold axes. Furthermore, the general direction of the strands within the β -sheet of STNV runs between the 5-fold and 3-fold axes⁵⁰, consistent with the respective strand direction in SBMV.

The close similarity between SBMV and the S domain of TBSV strongly supports divergence of these two viruses from a common ancestor. The possibility of an earlier gene duplication from which the P and S domains of TBSV have evolved is also possible in light of their structural similarity⁵¹. The possibility that not only the coat protein genes but also much of the rest of the SBMV and TBSV genome have a common ancestry must therefore be considered. The similarities shown here and by Argos¹⁰ between various groups of small spherical RNA viruses suggest that the β -barrel found in the SBMV subunit and TBSV S domain has been selected to comply with the functional needs of such viruses. The results of the structural studies of STNV⁵⁰, cowpea mosaic virus⁸ and belladonna mottle virus (P. Argos, personal communication) will, therefore, be awaited with much interest.

This work has been possible only through the dedication of many people over a period of nine years. In particular we thank Mrs Mary Ann Wagner, Dr Ira Smiley, Dr Toshio Akimoto, Mr Joel White, Miss Karen Babos and Mrs Kumiko Tsukihara, also Dr Patrick Argos for stimulating discussions, Jane Richardson for producing the diagrammatic representation of SBMV, Dr Steve Harrison for supplying the TBSV C_α co-ordinates, Dr Saul Rosen, Mr John Steele and Dr Victor Abell for support in computations, Drs Richard Lister and Andrew Jackson for advice on the growth of SBMV, Mr Eric Heness, the late Mr Philip Hamilton and Sharon Wilder for technical assistance. The work was supported by NIH grant AI 11219, NSF grant PCM78-16584 and a grant from the Lilly Company. D.S. was supported by a Deutsche Forschungsgemeinschaft Postdoctoral Fellowship and S.S.A., J.E.J. and I.R. by NIH postdoctoral fellowships 1 F32 GM 07103, 6 F22 CA 00557 and 1 F32 AI 05600, respectively, during part of this work.

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LETTERS

Cosmic X-ray background and intergalactic gas accretion onto intergalactic collapsed objects

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The origin of the diffuse cosmic X-ray background (CXB) has become the subject of much speculation recently, with most theorists divided between the intergalactic hot gas bremsstrahlung hypothesis and the QSO contribution theory. Stimulated by Sargent's recent argument about the existence of a cool, low density intergalactic gas, we investigate here the third possibility of its accretion onto intergalactic collapsed objects as the sources of the X-ray background. We conclude that if the CXB is hot thermal bremsstrahlung (TB) from the accretion of Sargent type intergalactic gas onto intergalactic collapsed objects, a new class of faint ($L_x \leq 10^{41} \text{ erg s}^{-1}$) low mass ($M \sim 10^7\text{--}10^{10} M_\odot$) discrete X-ray sources must exist with no optical or IR images, some of which could be detected by the Einstein observatory.

HEAO 1 observations have established that the cosmic X-ray background spectrum (3–50 keV) is most naturally fit by TB with $kT = 45 \pm 5 \text{ keV}$ (ref. 1). Yet the hypothesis that it may be emission from a hot intergalactic gas (IGG) of Ω_0 (present mean density/closure density) $\sim 0.5(\rho/\langle\rho^2\rangle^{1/2})$ faces severe difficulties, including the huge energy requirement, the destruction of H I clouds believed to be causing the quasar absorption lines, the Compton distortion of the microwave spectrum, and so on². On the other hand, source counts by the Einstein observatory indicate that faint sources down to $\sim 0.002 \text{ UFU}$ (Uhuru flux unit) already make up 30–40% (at least in the 1–3 keV band) of the CXB. If the present number-flux relation $N(>S) = KS^{n(1.5-1.8)}$ holds to much fainter S , then known discrete sources may easily account for all (or even exceed) the CXB intensity³, consistent with the conjecture that contribution from known optical QSOs brighter than $B \approx 20.3 \text{ mag}$ could account for essentially all of the CXB if L_x/L_{optical} remains large down to such magnitudes^{4,5}. Reasonable as such estimates seem, they remain unverified extrapolations at present, not to mention the highly uncertain nature of QSO X-ray spectra. Hence it is still interesting to investigate alternative sources for at least a significant fraction of the CXB.

Recently, Sargent *et al.*⁶ have proposed that the survival of the intergalactic H I absorption clouds favours the existence of an

IGG with $\Omega \sim 0.1$ and $T \sim 10^5 \text{ K}$ at $Z \sim 2.5$, too cool to produce the CXB. However, its existence raises the question of whether the CXB might be produced by accretion onto an intergalactic population of collapsed objects whose existence could not be detected otherwise. Such a hypothesis has some merits as (1) accretion onto compact objects is the most efficient mechanism known in X-ray production; (2) the low IGG density makes high temperature TB favourable and leads to low X-ray luminosity objects difficult to resolve. Here we investigate the constraints on such a model. The dominant contribution comes from the present epoch ($Z < 3$). Our work, therefore, complements that of Carr⁷ on pregalactic black holes ($Z > 10$).

A collapsed compact object of mass M moving with Mach number $\mathcal{M}$ in a fully ionized IGG of density ρ and temperature $T_4 10^4 \text{ K}$ (sound speed $c_s \sim 10^6 T_4^{1/2}$) will accrete at the Bondi rate⁸

$$\dot{M} \approx 10^{35} \text{ g s}^{-1} \left(\frac{M}{M_\odot} \right)^2 \rho T_4^{-3/2} \mu^{-3}; \quad \mu \equiv (1 + \mathcal{M}^2)^{1/2} \quad (1)$$

when the IGG turbulent velocity at distances $\geq GM/c_s^2$ is small compared with keplerian orbital velocity, which we assume. The X-ray luminosity from accretion is

$$L_x = \epsilon_x \dot{M} c^2 \approx 10^{56} \text{ erg s}^{-1} \left(\frac{M}{M_\odot} \right)^2 \epsilon_x \rho T_4^{-3/2} \mu^{-3} \quad (2)$$

where ϵ_x , the X-ray energy conversion efficiency is assumed M -independent. For $T_4 > 1$ the dominant cooling of IGG is adiabatic². Hence $T \propto (1+Z)^2$, $\rho \propto (1+Z)^3$ and L_x is epoch-independent provided ϵ_x is. For such non-evolving sources 80% of their contribution to the CXB comes from $Z \leq 1$ (ref. 9) and the volume emissivity needed to account for the observed CXB intensity is

$$\eta_x \approx 6 \times 10^{-35} \text{ erg cm}^{-3} \text{ s}^{-1} (1+Z)^3 \quad (3)$$

If most of the CXB comes from sources of mass M and number density N then equating $\eta_x = NL_x$ we have

$$N_0 \left(\frac{M}{M_\odot} \right)^2 \epsilon_x \rho_0 T_0^{-3/2} \mu_0^{-3} = 6 \times 10^{-91} \quad (4)$$

where all quantities with subscript '0' refers to $Z = 0$. In the model by Sargent *et al.*⁶, T drops faster than adiabatic after $Z \leq 2$ due to He-recombination cooling, leading to $T_0 \sim 10^3 \text{ K}$ (incomplete ionization) and contribution from $Z \leq 1$ greater than 80%. However, the uncertainties in other parameters are such that we can use equation (4) even in this case, lumping any correction into say, μ_0 . Rewriting $N_0 M = \Omega_B 4.7 \times 10^{-30} \text{ g cm}^{-3}$ and $\rho_0 = \Omega_0 4.7 \times 10^{-30} \text{ g cm}^{-3}$ ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) gives

$$\frac{M}{M_{\odot}} = 54 q^{-1}; \quad L_X = 1.2 \times 10^{30} \text{ erg s}^{-1} q^{-1} \Omega_B^{-1}$$

$$N_0 = 1.3 \times 10^9 \text{ Mpc}^{-3} q \Omega_B \quad (5)$$

where $q \equiv \epsilon_X \Omega_0 \Omega_B T_{04}^{-3/2} \mu_0^{-3}$ is expected to be $\ll 1$. Hence such objects must be supermassive black holes. These constraints can be generalized to a power law mass spectrum of compact objects, say $dN/dM \propto M^{-\gamma}$ for $M_1 \leq M \leq M_2$. Then the mass constraint in equation (5) applies approximately to M_2 for $\gamma < 2$ or M_1 for $\gamma > 3$.

The strongest constraint on the parameters in equation (5) comes from the sources count and fluctuation limit. From Uhuru and Ariel 5 small scale fluctuations ($\delta I/I \leq 2.5\%$ at 0.004 sr beam width), Schwartz⁹ already puts an upper limit on possible contribution of unknown classes of discrete sources to K (number of sources with $S > 1$ UFU per sr) < 10 . The Einstein survey gives $K \approx 7 \pm 3$ for all sources³, most of which have optical images. In the preliminary Draco and Eridanus surveys, only three out of 43 sources have empty optical fields³. Hence it seems reasonable to put a conservative upper limit for the contribution of accreting intergalactic black holes to the source count

$$K_B \leq 2 \quad (6)$$

Future observations may further lower this limit. As most such contributions would come from $Z \leq 1$ we have (see ref. 9):

$$K_B \approx 3.7 \times 10^6 L_{X44}^{3/2} N_0; \quad L_{X44} = L_X / 10^{44} \text{ erg s}^{-1} \quad (7)$$

Combining equations (5), (6) and (7) we have:

$$\epsilon_X^{1/2} \Omega_B \geq 3.2 \times 10^{-6} \Omega_0^{-1/2} T_{04}^{3/4} \mu_0^{3/2} \quad (8)$$

$$L_X \leq 1.2 \times 10^{41} \text{ erg s}^{-1} \quad (9)$$

$$M/M_{\odot} \leq 1.7 \times 10^7 \epsilon_X^{-1/2} \Omega_0^{-1/2} T_{04}^{3/4} \mu_0^{3/2} \quad (10)$$

$$N_0 \geq 1.3 \times 10^{-2} (\text{Mpc})^{-3} \quad (11)$$

For the IGG proposed by Sargent *et al.*⁶ with $\Omega_0 \sim 0.1$, $T_{04} \sim 1$ and $\mu_0 \sim 10$ we find $\Omega_B \geq 3 \times 10^{-4} \epsilon_X^{-1/2}$ and $M \leq 2 \times 10^9 M_{\odot} \epsilon_X^{-1/2}$.

If the IGG contains even a minute amount of organized angular momentum, the accretion flow will turn into a disk near the black hole¹⁰ where most of the X-ray is emitted, in which case ϵ_X could be high. The familiar disk model with optically thin TB emission has maximum temperature $T_e \sim 10(MM_{\odot}/M)^{1/2}$ provided $10^7 \text{ K} \ll T_e \leq 10^9 \text{ K}$ (ref. 11). If we set this to the observed CXB TB temperature we find (see equation (5))

$$\epsilon_X \Omega_B \sim 10^{-7} \quad (12)$$

When equation (12) is combined with equations (8) and (5) we get the constraints

$$\epsilon_X \leq 10^{-3} \Omega_0 T_{04}^{-3/2} \mu_0^{-3}; \quad \Omega_B \geq 10^{-4} \Omega_0^{-1} T_{04}^{3/2} \mu_0^3$$

$$\frac{M}{M_{\odot}} \sim 5 \times 10^8 \Omega_0^{-1} T_{04}^{3/2} \mu_0^3 \quad (13)$$

These numbers are uncertain to the extent the TB disk models are. Also, the requirement that the IGG is not depleted by accretion in a Hubble time demands $\epsilon_X \Omega_B \geq 10^{-8}$. The above results are summarized in Fig. 1.

We conclude that it is possible that much of the CXB is produced by the hot TB emission of Sargent type IGG accreting onto intergalactic black holes in the present epoch ($Z \leq 3$). This picture then predicts the existence of a new class of faint ($L_X \leq 10^{41} \text{ erg s}^{-1}$), numerous ($N_0 \geq 10^{-2} \text{ Mpc}^{-3}$) and low mass ($M \sim 10^7 - 10^{10} M_{\odot}$) discrete sources without optical or IR images. Gorenstein has pointed out that such objects may be separated from (optically) faint QSOs in the Einstein survey by studying the distribution of the lower limits to their L_X/L_{optical} ratio (P. Gorenstein, personal communication). Note that the bulk of such sources are fainter than the limit of the HR I of Einstein so their predicted $N \propto S^{-1.5}$ does not conflict with the observed steeper distribution. Note that these masses overlap with the mass corresponding to nonlinear density clumps

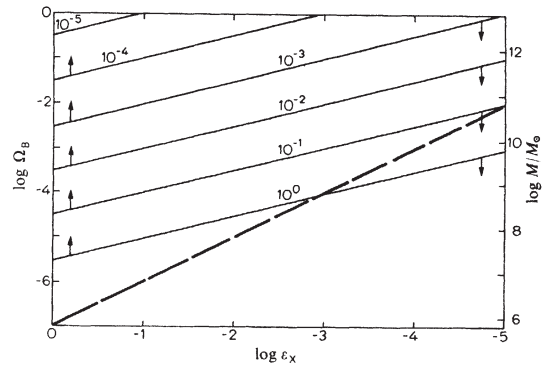


Fig. 1 Solid curves give the lower limit on Ω_B and upper limit on M as provided by the source count limit $K_B \leq 2$. The number on each curve labels the value of $\omega \equiv \Omega_0^{1/2} T_{04}^{-3/4} \mu_0^{-3/2}$ of the IGG (see equation (5)). The dashed curve represents the constraint on Ω_B for the TB disk model to give the observed CXB temperature. In this case M is independent of Ω_B or ϵ_X (see equation (13)).

($\delta\rho/\rho \sim 1$) at decoupling as extrapolated from the present power law density correlation function^{12,13}. Some of such clumps could (but need not) directly collapse into black holes without fragmenting into stellar systems at $Z \geq 10$ and their X-rays could have Compton-heated the denser IGG⁷. In such case the IGG would not evolve adiabatically and Carr finds that the direct contribution of accretion to the CXB at $Z = 10$ may also be significant⁷.

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Discontinuities in the jovian plasma disk of sulphur and oxygen ions

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The process of local acceleration of the plasma cloud freshly ejected from Io into the jovian magnetosphere is considered here. A physical model previously employed to investigate the motion of artificial barium cloud, is used to estimate the time scale for such transient acceleration. In normal conditions, the new ions could be accelerated to full corotation within $\sim 3 \times 10^2$ s of injection. But the corresponding time scale might be increased 10-fold or so at the peaks of mass injection. In this way the observations of non-rigid corotation of the S ions of sporadic nature may be understood. The temperature and ionization discontinuities in the Io torus as observed by the Voyager experiments is also considered. Here we propose that such a sharp transition is related to the coupling of the radial diffusion and heating plus cooling of the thermal plasma.

Ground-based observations¹⁻⁶ indicate that the S optical emissions of the jovian sulphur nebula at 6,717 and 6,730 Å usually have a very sharp cutoff at, or inside of, the orbit of Io at 6 R_J , whereas the inner boundary between 3 and 4 R_J has a diffuse appearance. At the same time, it has been established that the motion of the sulphur ions in this nebula is in corotation with the jovian magnetosphere¹. Although these descriptions may be considered to represent the norm of the S⁺ plasma disk, Mekler *et al.*⁷ have recently presented evidence of occasional weak S⁺ emission outside the orbit of Io. Particularly interesting are the velocities of the S⁺ ions in this anomalous emission. Although the motion of the S⁺ plasma inside of the sharp boundary was in rigid corotation, the corresponding motion in the outer emission was slower than would be expected for corotation. Such unusual phenomena may be caused by some disturbances in the jovian magnetosphere (for example, substorms), or simply related to the injection process of the sulphur ions from Io. [Note that evidence of non-rigid corotation (up to a few per cent) of the jovian magnetospheric plasma in the vicinity of the orbit of Io have been recently reported^{8,9} on the basis of plasma wave and planetary radio astronomy experiments. These observations are presumably more representative of the quiescent state of the Io torus.]

A model formulated by Scholer¹⁰ to study the motion of ionized barium cloud artificially released in the Earth's magnetosphere^{11,12} will now be applied to the case of the Io torus of sulphur and oxygen ions. Although highly simplified, this treatment gives a reasonable approximation to the MHD transfer of angular momentum from Jupiter to the Io torus and the resultant heating of the ion cloud. This model together with considerations on the radial diffusion and cooling of the ion cloud, and the new information from the Voyager 1 and 2 observations of the jovian system¹³⁻¹⁸, would allow us to interpret systematically the non-corotation events and several other related observations.

The geometry of the MHD coupling between the Io torus and the jovian ionosphere will first be represented by a two-dimensional system with both the ionosphere and the ion cloud idealized as flat slabs of finite vertical thickness and width in the y -direction, and the extension in the x -direction is supposed to be infinite, see Fig. 1. The planetary magnetic fields of uniform strength B are pointing in the z -direction. Furthermore, the magnetosphere between the ionosphere and the Io torus is assumed to have a plasma density of ρ , while the surface density of the torus is m_0 (in g cm⁻²). The motion of the torus is then coupled to that of the ionosphere through the continuous transmissions and reflections of the Alfvén waves propagating between these two regions¹⁹ with acceleration of the ion cloud as the end result.

More specifically, as the waves travel through the magnetosphere with the Alfvén speed $V_A = B/(4\pi\rho)^{1/2}$ there will be a polarization current j_p carried at the wave fronts^{10,20-23}. As shown in Fig. 1a, the current will be closed in such a way that the $\mathbf{j} \times \mathbf{B}$ forces would decelerate the magnetospheric, or the ionospheric, plasma but accelerate the ion cloud. The total polarization current can be expressed as $I_p = \sum_A E \uparrow$, where $E \uparrow$ is the electric field applied at the (upward going) wave front and $\sum_A = c^2/4\pi V_A$ is the so-called wave conductivity of the magnetosphere. As the wave reaches the ionosphere with a height-integrated Pedersen conductivity $\sum$, an electric field $E \downarrow$ will be reflected, whereas a conduction current $I_1 = \sum(E \uparrow - E \downarrow)$ will be set up flowing across the magnetic field.

As the ionospheric conduction current must be equal to the total current in the magnetosphere, that is:

$$\sum(E \uparrow - E \downarrow) = \sum_A(E \uparrow + E \downarrow) \quad (1)$$

we have the following relationship between the incoming and reflected waves:

$$E \downarrow = \left[\frac{1-X}{1+X} \right] E \uparrow \quad (2)$$

with $X = \sum/\sum_A$. Thus, the reflection coefficient of the Alfvén waves at the ionospheric boundary is given by $\alpha = (1-X)/(1+X)$. From the same reasoning we can see that after a time $T_A = 2L/V_A$, which is the characteristic time for an Alfvén wave to travel back and forth between the ion cloud and the ionosphere, there will be another reflection, and so on. The acceleration of the ion cloud is, therefore, not just a function of the ratio between $\sum$ and $\sum_A$ but also time t . Another important factor in this dynamical problem is the distributed mass of the ion cloud. Assuming that the central slab is initially at rest (which is pertinent to the case when an ion cloud is just ejected from Io) and taking the inertia of the ion cloud into account, a general solution for the velocity of the cloud can be derived as was done by Scholer¹⁰ for the barium cloud release experiments. In the present context we have

$$V(t) = V_0 \left\{ 1 - \sum_{n=0}^{\infty} (-1)^n \alpha^n [\exp(-t_n/T_0) L_n(2t_n/T_0) + \alpha \exp(-t_{n+1}/T_0) L_n(2t_{n+1}/T_0)] \right\} \quad (3)$$

Here $t_n = t - nT_A$, $T_0 = 2m_0 V_A/B^2$, V_0 is the corotation speed, and the summation is carried out only for the Laguerre polynomials $L_n(t_n)$ with $t_n \geq 0$. The normalized velocity profiles for various combinations of T_0 , T_A , and α are shown in Fig. 2.

Briefly, for $\sum \gg \sum_A$ and hence $\alpha \approx -1$, the MHD coupling between the ionosphere and the ion cloud is very strong, and in the case of small T_A/T_0 ratio the ion cloud will be accelerated to full corotation speed in just a few times of T_A . On the other hand, if $\sum \ll \sum_A$ and hence $\alpha \approx 1$, the motion of the ion cloud is only weakly coupled to the ionosphere by Alfvén waves. Acceleration of the ion cloud would be very slow. The inertia effect of the ion cloud can also be observed by examining the velocity profiles with different T_A/T_0 ratios but the same value of α .

To explore this result further we assume, for example, the magnetospheric plasma density to be ≈ 1 proton cm⁻², the surface number density n_s of the ion cloud just ejected from Io to be $\approx 2 \times 10^{12}$ ions cm⁻², and the length scale L to be $\approx 10R_J$; the

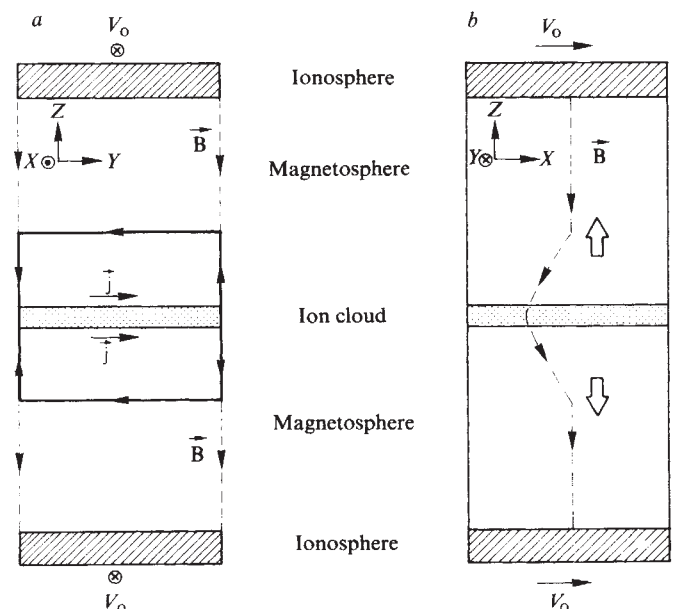


Fig. 1 a, A cross-section of the model in the Y - Z plane. The wave current system decelerating the magnetosphere and accelerating the ion cloud is sketched. As a result of the successive transmissions and reflections of these guided waves, a field-aligned current system will be established between the ionosphere and the Io cloud, similar to the current system detected in the vicinity of Io^{21,40-43}. The auroral hiss observed on the inner edge of the torus at $L \approx 5.6 R_J$ (ref. 43) is probably caused by the beaming of low energy electrons (10 eV–1 keV) associated with such current flows. b, A finite section of the model in the X - Z plane. The upward and downward propagations of the Alfvén waves are indicated.

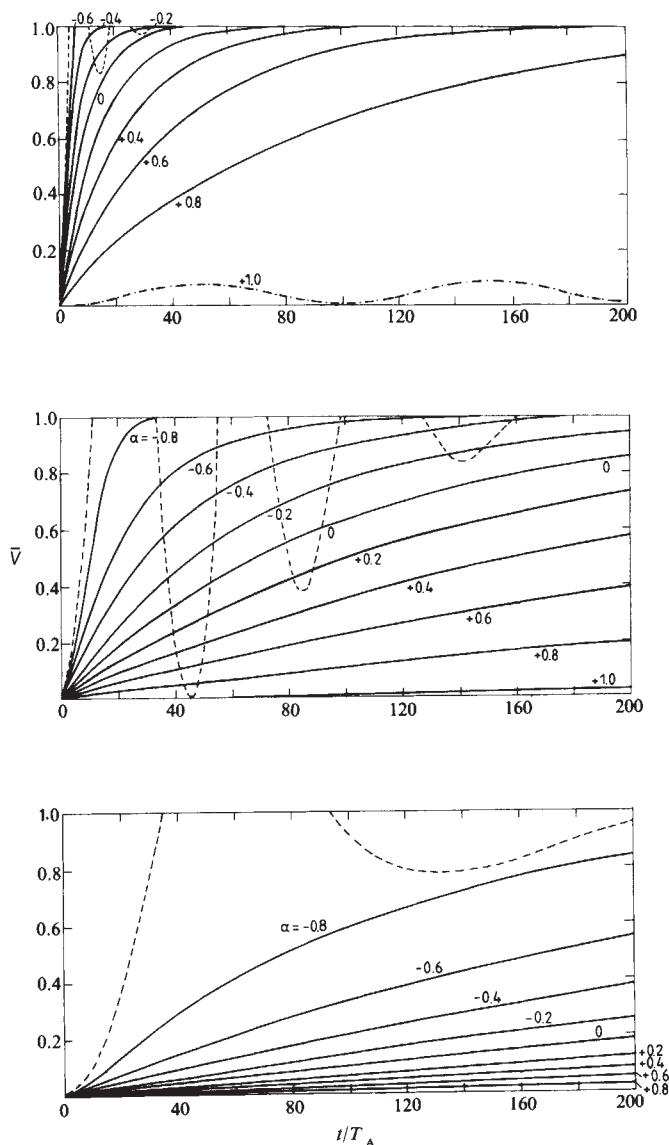


Fig. 2 Velocity profiles of the ion cloud (initially at rest) normalized to the corotation velocity V_0 for various values of the T_A/T_0 ratio and the reflection coefficients α . For $\alpha \leq -0.6$, $\sum \geq 4\sum_A$. If the plasma density in the magnetosphere is 1 proton cm^{-3} , and $B = 2 \times 10^3$ nT we have $\sum_A \geq 0.02$ S, consequently $\sum \geq 0.1$ S. In this MHD approximation, the ion cloud velocity oscillates before reaching the corotation velocity for $\alpha \rightarrow -1$ (see dashed lines). A more realistic description is for the ion cloud to be accelerated to the full speed without such overshoots. *a*, $T_A/T_0 = 10^{-1}$; *b*, $T_A/T_0 = 10^{-2}$; *c*, $T_A/T_0 = 10^{-3}$.

value of the ratio T_A/T_0 can then be approximated to be ≈ 0.1 . (As the total ejection rate can be written as $\dot{N} \approx 2n_0R_sV_0$, $\dot{N} \approx 5 \times 10^{27}$ ions s^{-1} if $n_s \approx 2 \times 10^{12}$ and R_s is the radius of Io. This value is compatible with the production rate of the sulphur and oxygen ions as estimated by Broadfoot *et al.*¹⁵.) As the S^+ nebula was generally observed to be in rigid corotation with the jovian magnetosphere, the characteristic time scale for the corresponding acceleration of the ion cloud probably is no more than a few times of T_A . Thus the α value may be taken to be ≤ -0.6 , or $\sum/\sum_A \geq 4$, say. With $T_A \approx 30$ s and $V_0 = 57$ km s^{-1} , Fig. 2 shows that the ions picked up directly from the ionosphere/exosphere of Io will be accelerated to full corotation within 10 Io radii. This trail of slow moving ions may be interpreted as the wake of the satellite ionosphere²⁴⁻²⁶. (This picture would still hold qualitatively for the interaction of Io with the torus of dense hot plasma.)

Now, if the ejection rate N were occasionally increased by a factor of 10 or so (for example, at the maximum of volcanic activities if ionospheric loss is the dominant injection process), $T_A/T_0 \leq 0.01$ and the resulting acceleration time scale will be lengthened by a similar factor. Thus, after its first ejection from Io, the ion cloud would probably stay behind corotation for at least an hour, leaving an ion wake $\sim 150^\circ$ long. This non-corotation effect could persist even longer if the volcanic activity lasts more than a few hours. The large mass of the fresh S^+ and O^+ ions injected in these events, of course, would enable the non-rigid corotation behaviour to be discerned more easily, especially outside the nominal edge of the S II nebula.

If the non-corotation of the weak emission of S^+ ions observed outside the nominal outer edge⁷ could be explained by the inertia effect of the MHD acceleration process plus the sporadic outbursts of the volcanic activities, how do we explain the simultaneous corotation of the S^+ ions observed in the inner region of stronger emission? And above all, why should there be a sharp discontinuity confining the S^+ emission within $6R_J$ in the first place. These questions may be answered by referring to the Voyager observations of the Io torus. First, the EUV experiment¹⁵ and the plasma experiments¹⁶ have found that the bulk of the heavy ions is apparently in the form of S^2 , S^3 , and O^{2+} with thermal temperature $kT \approx 5-50$ eV. This hot ion torus—with its point of maximum number density approximately located at $5.8R_J$ —has $5R_J$ as its inner boundary and $7-8R_J$ the outer boundary. Second, a sharp drop in temperature from $kT \approx 10$ eV to 1 eV was found at $5.7R_J$ by the plasma experiments¹⁶. Together with the temperature change, there is also an apparent change in the ionization state of the heavy ions from S^{2+} , O^{2+} to S^+ and O^+ . Such stratification of the plasma temperature and ionization might be related to the balance between ionization equilibrium and thermal equilibrium as was found in the structures of planetary nebulae²⁷⁻²⁹. Note that the ions freshly created in the vicinity of Io can have kT as high as 260 eV for O^+ and 520 eV for S^+ (ref. 30) the maximum time scale for cooling of these new ions³¹ can be calculated to be $t_c \leq 1.5 \times 10^5$ s. In comparison, adopting a radial diffusion coefficient of $10^{-6} R_J^2 \text{s}^{-1}$ (ref. 32), the time scale for diffusion across a distance of $0.5R_J$ at this region is $t_d \approx 7 \times 10^4$ s. As $t_d \geq t_c$, the heating of the Io torus is then limited to a distance of less than $0.5R_J$ around the orbit of Io. Although this may partly explain the sharp division between the hot and cold plasma at $5.7R_J$, issue could be raised about the absence of such discontinuity at $6.3R_J$ or so. An explanation of such asymmetric behaviour may come from the additional heating effect beyond $6R_J$ as a result of Landau damping of the ion cyclotron waves, for example (ref. 33 and C. K. Goertz, personal communication). We may argue that the energetic (S^+ and O^+) ions, which are responsible for generation of the ion cyclotron turbulence, would not be able to diffuse across Io due to its absorption effect; and this in turn would limit the plasma wave activities and hence plasma heating to radial distance $> 5.9R_J$.

The ionization state and thermal condition of the Io plasma disk is therefore determined by four inter-related time-dependent processes, namely: (1) the ejection rate; (2) the MHD acceleration; (3) the radial diffusion; and (4) the cooling and heating of the ion cloud. Not just the structure of the 'quietest' S^+ nebula and the non-corotation of the anomalous emission during the 'disturbed' periods, but also the temperature and density discontinuities together with the apparent ionization stratification in the Io torus can be explained in this way.

While we have concentrated on the local and transient effect acceleration of the plasma just ejected from Io, clearly the time-averaged acceleration and transfer of angular momentum of the Io torus in a more global scale must be of the same vein. For a description of the non-rigid corotation of the jovian magnetospheric plasma at radial distances beyond $6R_J$, see refs 34-36. Alternative views on the asymmetric radial distribution of the thermal plasma^{37,38} addressing the possibility of two different diffusion mechanisms at both sides of Io had also been proposed. The effect of such process on our hypothesis of the temperature and ionization discontinuity remains to be investi-

gated. The recent report by Shemanski that an upper limit of 10^{27} ions s^{-1} may be derived for the ion source in the vicinity of Io is also problematic as it sets a stringent condition on the model of local mass pick up^{25,26}. In any event, our primary result for the transient acceleration of the Io plasma cloud is by no means affected because, in principle, it is valid for both local (ionospheric scavenging) and more extended (ionization of the sputtered atoms) ion sources.

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S-wave anisotropy in the upper mantle under a volcanic area in Japan

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Most ultra-basic nodules that probably originated in the upper mantle have anisotropic petrofabric and elastic properties^{1,2}. Within seismology, however, the Earth is generally assumed to be isotropic and this assumption is appropriate for most seismological studies. Thus, rocks can be considered isotropic on large scales although anisotropic on a scale of hand specimen³. This interpretation is questionable, however, in light of the increasing resolution of seismic observations. Many reliable reports on seismic anisotropies have been accumulating: for example, azimuthal anisotropy in P_n velocities in oceanic basins⁴, and in the continent⁵ and the back-arc basin⁶, and an S-wave splitting of the SH and SV waves revealing an anisotropic structure as deep as 190 km (ref. 7). These suggest that some unsuccessful detections of the Earth's anisotropies may be due to lack of appropriate observations. We describe here the recent discovery of shear wave anisotropy in a volcanic area in Japan, which was revealed by an improvement in the seismographic observation systems.

Telemetry systems of the seismographic stations have recently been established by Kyoto and Nagoya Universities⁸ (Fig. 1) in the Chubu area, Japan, beneath which the Pacific plate is subducting. This instrumental development improves the spatial coverage, timing accuracy and recording conditions of seismic observations. The Kamitakara Observatory of Kyoto University is located in the northern part of this area⁹ (Fig. 1). Immediately beneath the observatory (Takayama area), at a depth of ~260 km (ref. 10) there are often deep earthquakes.

These are very useful events for studies of structures within the wedge between the subducting plate and the Earth's surface in the island arc. As seismic ray paths from these events to the seismographic stations are almost vertical, the wave forms are quite simple because of little contamination of converted waves generated at conspicuous layer boundaries. The stations AMJ and KTJ of this observatory are equipped with the three-component seismographs and the station NRJ with a vertical component one only. The natural pendulum period T_0 is 1 s for both vertical and horizontal seismographs.

Figure 2a shows seismograms of one of those deep earthquakes (the earthquake 1 in Fig. 1 and Table 1) recorded at the Kamitakara Observatory. Because the P-wave arrives at the three stations within 0.2 s, the incidence angle is less than 5°. Remarkably, the horizontal seismograms show two distinct arrivals of the shear wave, a fast arrival for the NS component and a slower arrival for the EW one. The horizontal seismograms of three more earthquakes from the same area (earthquakes 2, 3 and 6 in Fig. 1 and Table 1) were examined. These seismograms showed the two different arrivals of the S-wave similar to that in Fig. 2a. Such differences in the arrival time of the S-wave between the two horizontal components would be caused by interference of converted waves, but for the present case this interpretation should be dismissed. No significant signal is in fact observed on the vertical component in Fig. 2a. A probable interpretation is that S-wave splitting is caused by anisotropy of the medium at intermediate locations between earthquake source and seismographic stations. The incident S-wave is generally resolved into two shear waves that travel along the same path with different velocities—a phenomenon known as acoustic 'double refraction'¹¹.

As the directions of horizontal seismographs, NS and EW, are not necessarily those of the principal axes of anisotropy, the two split shear waves are generally not resolved on the two horizontal seismograms. Figure 2a implies, however, that the directions of the principal axes of the anisotropy are rather close to NS and EW. To determine precise directions of the velocity axes, we synthesized the S-wave forms, by rotating the two orthogonal axes in a clockwise direction at an interval 15°, and projecting the observed S-wave particle motion onto the rotated axes. When a rotation angle θ from the north or east coincided with a principal axis, the two orthogonal S-waves became most widely separated. As shown in Fig. 2b, $\theta = 15^\circ$ seems to provide the clearest separation for both records of AMJ and KTJ. The azimuth of maximum velocity, thus, is N15°E and the azimuth of minimum velocity is E15°S.

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Table 1 A list of earthquakes used for this study and their anisotropic parameters

No.	Date (JST)	Depth (km)	KTJ			AMJ			KAJ			IMJ			TAK			ITD			TKC			INU			UGK		
			D	A _z	Q	D	A _z	Q	D	A _z	Q	D	A _z	Q	D	A _z	Q	D	A _z	Q	D	A _z	Q	D	A _z	Q			
1977																													
1	25 Sep	260	0.8	15°	A	1.0	0°	A	0.0	0°	A																		
2	7 Oct	260	0.8	20°	A	0.3	0°	B																					
3	18 Nov	275	0.8	40°	A	0.9	15°	A																					
4	30 Nov	360	0.0	0°	A	0.0	0°	A																					
1978																													
5	1 Feb	240	0.0	0°	B	0.0	0°	B																					
6	9 Mar	260	0.7	30°	A	0.5	15°	C																					
7	5 Apr	260							0.3	105°	B																		
8	27 May	280	0.9	15°	A	?		C				0.8	15°	A	0.0	0°	A												
9	14 Jun	360	0.0	0°	B	0.0	0°	A				0.0	0°	A	0.0	0°	A	0.0	0°	B				0.0	0°	A			
10	27 Jun	200	?			0.9	30°	B																					
11	3 Aug	230	0.0	0°	A	0.9	30°	B																					
12	14 Aug	280													0.3	90°	B				0.0	0°	B						

D, The arrival time difference between the two shear waves in s. A_z, The azimuth of the vibration with the maximum velocity in deg. Q, the quality of the data.

To constrain the size and location of the anisotropy, we collected more data which were recorded at neighbouring seismographic networks, the Hokuriku Observatory of Kyoto University (7 stations), and Nagoya University (10 stations) (Fig. 1). The seismographs of the Hokuriku Observatory are the same type as in the Kamitaka Observatory and the two-horizontal-component-observation was carried out at either KAJ or IMJ. In the large seismographic network of Nagoya University, two horizontal-component seismographs are installed at all the stations ($T_0 = 10$ s for both vertical and horizontal components). In each network, vertical seismograms were recorded continuously on paper, but horizontal seismograms were stored on only magnetic tapes when signal amplitudes exceeded a threshold value.

A clear split of the S-wave of earthquake 1 was also recorded on horizontal seismograms at TAK of Nagoya University, 20 km away from KTJ (Fig. 2c). The long-period nature of the seismographs of Nagoya University well suppresses short-period noises. The same procedure as described above was used to determine the anisotropic parameters for the Nagoya seismograms. The arrival time difference was 0.7 s, and the directions of the maximum and minimum velocity axes were N15°E and E15°S, respectively (Fig. 2c). The polarization angle of the S-wave's first motion corrected for the differential arrival is consistent with the P-wave's nodal plane solution. The consistency of the anisotropic parameters between the two indepen-

dent networks is further evidence for the existence of the anisotropy under Takayama.

Twelve earthquakes were selected between September 1977 and October 1978; 28 ray paths were used (Table 1). Most of the earthquakes originated from the source 260 km beneath Takayama. To each S-wave signal, the same procedures represented in Fig. 2b, c, d were carried out. The azimuth of the principal axis of the maximum velocity from the north, the arrival time difference between the two S-wave components, and the quality of the data (A, B and C from high to low) are listed in Table 1. Data with quality C were not used for the estimation of the arrival time difference. To avoid corruption of S-wave form by converted waves, we restricted our study to emergence angles <20° at the hypocentre. Because each network has its own amplitude threshold for the start of magnetic tape recording and individual transmission-recording characteristics, the number of earthquakes available for this study differs among the networks. As the coverage of ray paths so far has been concentrated primarily under Takayama and its vicinity, we limit our discussion of anisotropy to that area.

The results are given in Table 1 and can be summarized as follows: (1) the S-wave splitting is observed at AMJ, KTJ and TAK for the earthquakes immediately beneath these stations, but not observed at ITD, TKC, IMJ and KAJ away from these stations; (2) the S-wave splitting is, on the other hand, not

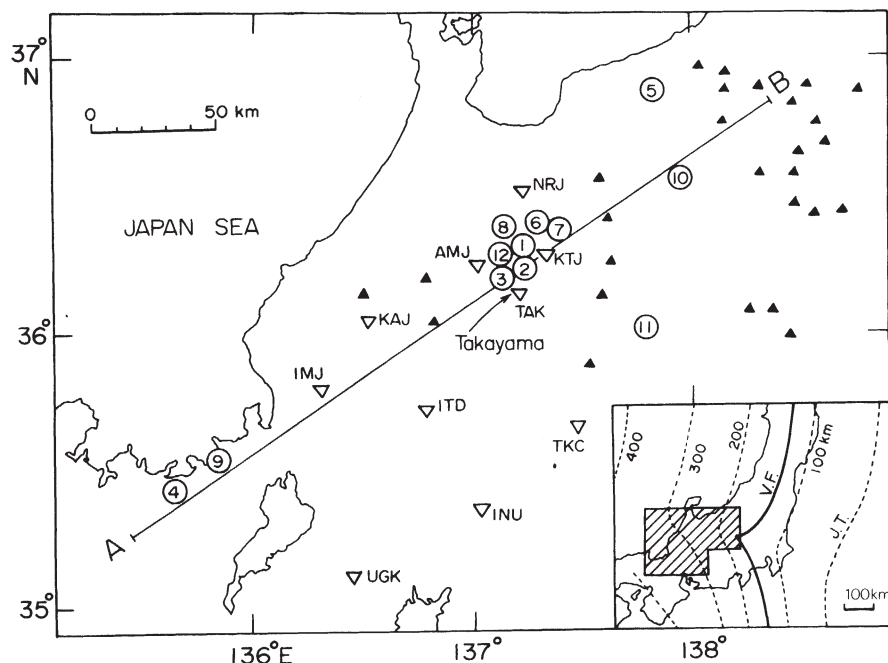


Fig. 1 Epicentres (○), seismographic stations (▽) and Quaternary volcanoes (▲) in the Chubu area, Japan. Numerals denote epicentres of the earthquakes corresponding to Table 1. Quaternary volcanoes are taken from Isshiki *et al.*¹⁷. The insert shows depths of earthquakes on the deep-focused seismic plane from Utsu¹⁸, and the volcanic front which forms the trenchward bound active volcanism¹⁶. Line A-B refers to Fig. 3.

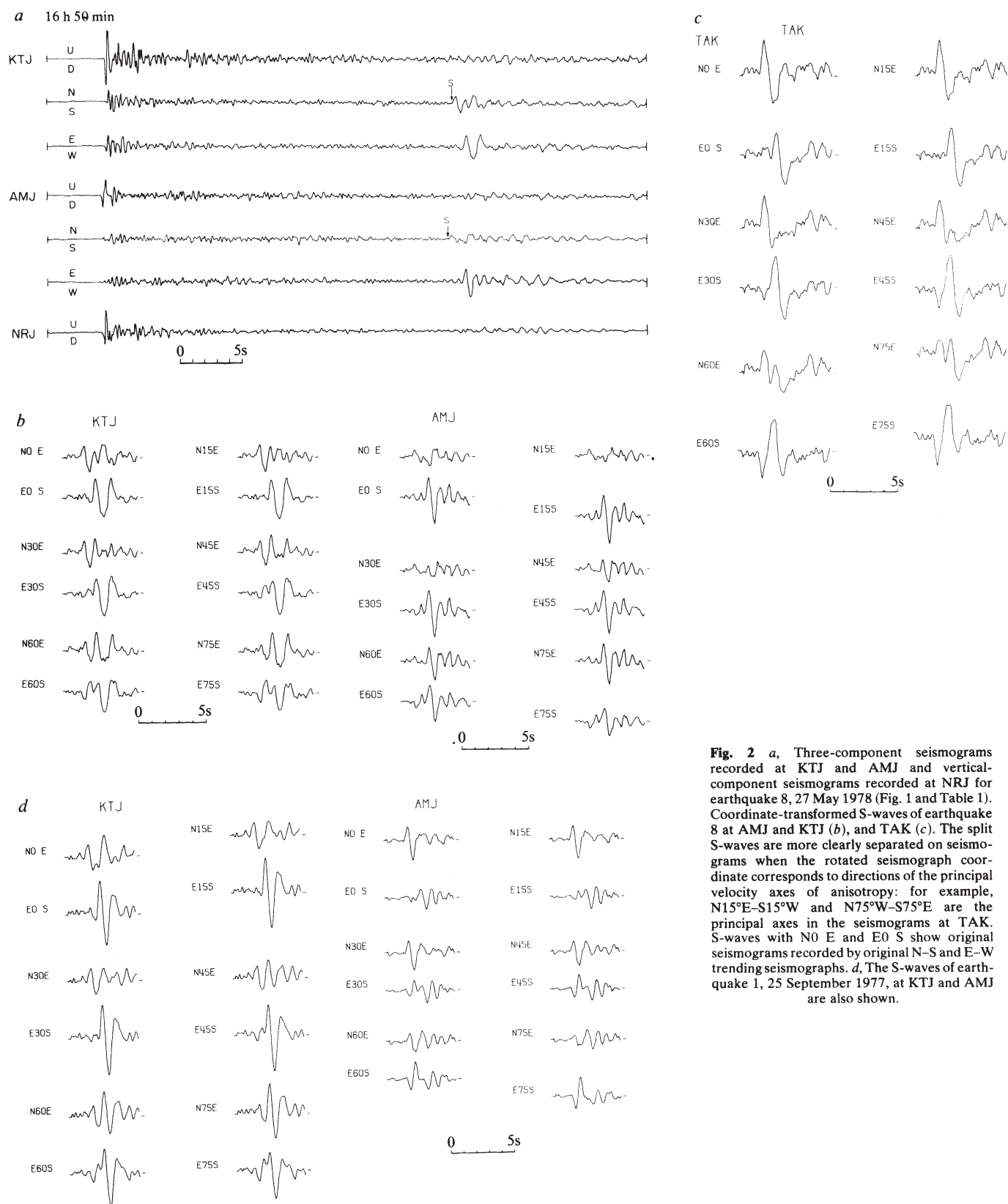


Fig. 2 *a*, Three-component seismograms recorded at KTJ and AMJ and vertical-component seismograms recorded at NRJ for earthquake 8, 27 May 1978 (Fig. 1 and Table 1). Coordinate-transformed S-waves of earthquake 8 at AMJ and KTJ (*b*), and TAK (*c*). The split S-waves are more clearly separated on seismograms when the rotated seismograph coordinate corresponds to directions of the principal velocity axes of anisotropy: for example, N15°E–S15°W and N75°W–S75°E are the principal axes in the seismograms at TAK. S-waves with N0°E and E0°S show original seismograms recorded by original N–S and E–W trending seismographs. *d*, The S-waves of earthquake 1, 25 September 1977, at KTJ and AMJ are also shown.

observed at AMJ, KTJ and TAK for deep earthquakes which occurred outside the Takayama area. These results suggest that the anisotropic medium is not distributed near the source or the receiver station, but rather at some intermediate location along the ray path as shown in Fig. 4. These arguments would be consistent with an anisotropic body ~50–100 km in both horizontal and vertical extents at depths ~100–200 km. The

corresponding velocity difference between the two shear waves is ~2–4%. The travel times of the S-wave with the faster velocities associated with the splitting are identical to those of S-waves from the standard travel timetable of the Japan Meteorological Agency¹². The travel times associated with the minimum velocities are thus delayed ~1 s relative to the standard time.

Preferred orientation of microcracks or liquid inclusion, or individual minerals may cause such an anisotropy of the S-wave^{11,13-15}. At present, it is difficult to interpret the anisotropy. We, however, hypothesize that this anisotropy is related to a magma reservoir because AMJ, KTJ and TAK are located near volcanoes inside the volcanic front¹⁶ (Fig. 1). It seems possible, therefore, that S-waves from the deep earthquakes pass through deep magma reservoirs beneath volcanoes (Fig. 3). If thin elliptical inclusions trending north-south exist within magma reservoirs, then a velocity anisotropy of S-waves could be caused with the maximum velocity axis NS.

Restrictions on the compositions of mid-ocean ridge basalts: a fluid dynamical investigation

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The nature of the melts parental to mid-ocean ridge basalts has been vigorously debated. Opinions vary between those considering the parental magma to be magnesia-rich (picritic basalt) with 15–20% MgO and those arguing that the parental melts are compositionally related to the most primitive liquids erupted at mid-ocean ridges (MgO ~ 9–11%). This difference of between 5 and 11% in MgO content represents a temperature interval of 100–250 °C. The actual MgO content places constraints on the thermal conditions at the mantle source. The high-magnesia liquid theory is supported by melting experiments on mantle and basalt samples¹⁻³ and by petrological studies of ophiolite complexes⁴. However, this theory does not seem to be immediately consistent with the fact that no glasses or aphyric rocks of these compositions have ever been sampled from the sea floor. Indeed the most primitive glass compositions that occur as inclusions in olivine megacrysts and as glassy margins to pillow lavas have MgO contents in the range 9–11%. Models of generating low MgO partial melts from the mantle have been proposed. For example, Presnall *et al.*⁵ consider that melting at cusps in the peridotite mantle solidus could generate such melts. We outline here a fluid dynamical model for a basaltic mid-ocean ridge magma chamber supplied by high-magnesia melt from the mantle. The model predicts that high-magnesia extrusives will not be erupted, that ultramafic cumulates should occur at the base of the oceanic crust, that lava compositions will be restricted to MgO contents <10% and that magma mixing will be a common feature of sea-floor basalts⁶.

Before presenting the model we emphasize a fundamental feature of the moderate to low pressure evolution of picritic to tholeiitic basalt by crystal fractionation⁷. Figure 1 shows the dependence of density of high-magnesia melts on MgO content as crystallization occurs, calculated by the method of Bottinga and Weill⁸. As an example, we use the estimate of Elthon⁴ for the parent magma for the oceanic crust (MgO ~ 17.8%) and generate successive residual liquids by crystallization of olivine (Fo₉₀). As olivine crystallizes out the density of the residual liquid decreases despite the declining temperature. The residual liquid reaches the density minimum A and the liquid joins a cotectic (perhaps olivine-plagioclase or olivine-plagioclase-pyroxene). Subsequent crystallization, dominated by plagioclase and pyroxene, generates residual liquids of progressively increasing density, due to iron-enrichment accompanied by low degrees of silica enrichment. Similar relationships between density and composition have been documented from several suites of mid-ocean ridge basalts⁷, although the exact position of the minimum point A may change slightly from case to case. The variation of magma density with composition is complicated by the effects of suspended crystals, but in general the effect of suspended crystals will accentuate the relationships shown in Fig. 1 (ref. 7) by increasing the magma density at a fixed temperature.

It is significant that the most primitive glasses yet recovered from the sea floor occupy the density minimum shown in Fig. 1 (9–11% MgO)^{6,7,9,10}. Mid-ocean ridge basalts are all more

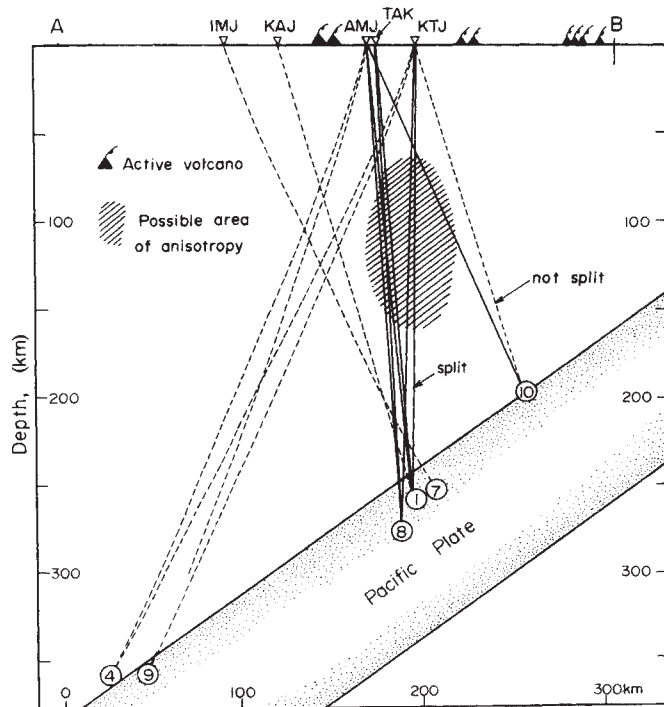


Fig. 3 S-wave ray paths for earthquakes 1, 4, 7, 8 and 10 projected on the plane A-B (Fig. 1). Solid rays denote those associated with the S-wave splitting (arrival time difference >0.4 s). A possible area of anisotropy is inferred from a combination of these ray paths.

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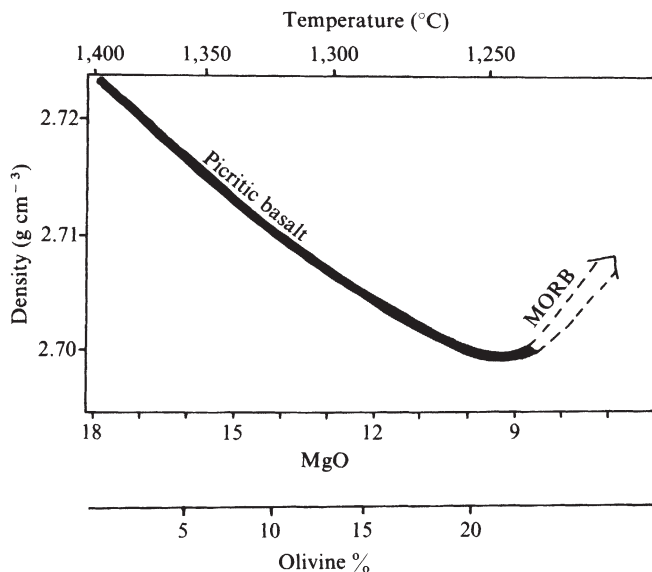


Fig. 1 Variation of melt density with MgO content in picritic liquids. The estimate of Elthon⁴ is used as an example of the parental magma to the ocean crust with MgO ~17.8% and generated a succession of liquid compositions down to MgO = 10% by removal of forsterite (Fo₉₀). The approximate percentage of olivine removed in generating the successive liquids is indicated by the lower scale. Liquidus temperatures were estimated approximately from the experimental data of Krishnamurthy and Cox¹⁷. The density minimum at A is illustrated with the approximate trend of mid-ocean ridge basalt density with MgO content for MgO < 9% (after ref. 7).

evolved than this composition and show abundant evidence for mixing between evolved liquids and more primitive liquids (with MgO 9–11%)^{6,9–11}.

Figure 2 shows our model of a magma chamber containing typical mid-ocean ridge basalt into which a new influx of hot, but denser, picritic-basalt melt is intruded. The concept of an open-system magma chamber periodically replenished by new melt from depth¹² is now widely accepted as the most probable situation existing beneath the mid-ocean ridges, although the exact geometry and location of mid-ocean ridge magma chambers is still uncertain¹³. The temperature contrast between the two magmas may be 100–200 °C, depending largely on the MgO content of the picritic basalt. Investigations of an analogous system¹⁴ where a hot salty layer of water underlies a cold freshwater layer indicates that the dense fluid will remain at the base of the chamber. Between the two layers there is a non-turbulent interface across which heat and salt are transferred by molecular rather than turbulent processes. Unless the densities of the two layers are virtually identical, no physical mixing occurs across the interface, although each layer convects vigorously. We suggest that this form of motion can also occur in magmas where the ratio of diffusivity of mass to that of heat is typically < 10⁻⁵.

We have adapted the empirical and theoretical results of studies on the salt/water system to the picrite/basalt system in a magma chamber. A detailed treatment of the problem, which has several geological implications in addition to the situation described here, will be presented elsewhere. We summarize here the results pertinent to the problems of mid-ocean ridge magma genesis. Vigorous convection occurs in the two layers (Fig. 2b) and heat transfer through the interface between the two layers results in rapid cooling of the lower layer.

Our calculations indicate that the temperature of the lower layer as a function of time, $T(t)$, can be expressed as

$$T(t) = T(0) + \left[\frac{\Delta^{-7/3} + 7A(0.5+r)t/3}{\Delta} - \Delta \right] / (1+2r)$$

where $T(0)$ is the initial temperature, Δ is the initial temperature

difference between the layers and r is the ratio of the thickness of the lower layer to that of the upper layer. The expression takes account of the heat of crystallization for olivine using a value of 200 cal g⁻¹. The constant A is dependent on the physical properties of the fluids.

$$A = 0.32(g\kappa^2\alpha^7/\nu)^{1/3}/(h\sigma^2)$$

where g is the gravitational acceleration, κ is the thermal diffusivity, α is the coefficient of thermal expansion, ν is the kinematic viscosity, h is the thickness of the lower layer and σ is the density contrast between the layers due to the difference in MgO content divided by the mean density.

Typical values of the physical properties are: $\kappa = 10^{-6}$ m² s⁻¹; $\alpha = 5 \times 10^{-5}$ K⁻¹; $\nu = 10^{-3}$ m² s⁻¹; $\sigma = 2 \times 10^{-2}$. Figure 3 shows the cooling history of the lower layer for values of h between 3 and 300 m with an upper layer thickness of 4 km, an initial upper layer temperature of 1,255 °C and an initial lower layer temperature of 1,385 °C. We observe that the lower layer cools to thermal equilibrium with the upper layer in a time scale of a few months to a few years. The reason for the rapid cooling rates is the intense turbulent convection that occurs for large Rayleigh numbers¹⁴. Typically, the initial Rayleigh number of the lower layer exceeds 10⁹.

Another consequence of the turbulently convecting flow regime is that olivines precipitated from the lower picrite layer will be kept in turbulent suspension throughout the cooling of the lower layer. In all plausible models we calculate that the mean turbulent velocities¹⁵ are in the range 1–5 cm s⁻¹, whereas the free-fall velocities of olivine crystals of 1–5 mm diameter (typical dimensions in ultramafic cumulates and olivine-phyric lavas) in magnesia-rich liquids are in the range 10⁻²–10⁻³ cm s⁻¹. The olivine settling rates only become comparable to the turbulent velocities when the temperature difference becomes small (< 1 °C).

The model predicts (Fig. 2) that the lower picritic basalt will not mix with the upper basalt layer and that it will cool rapidly in

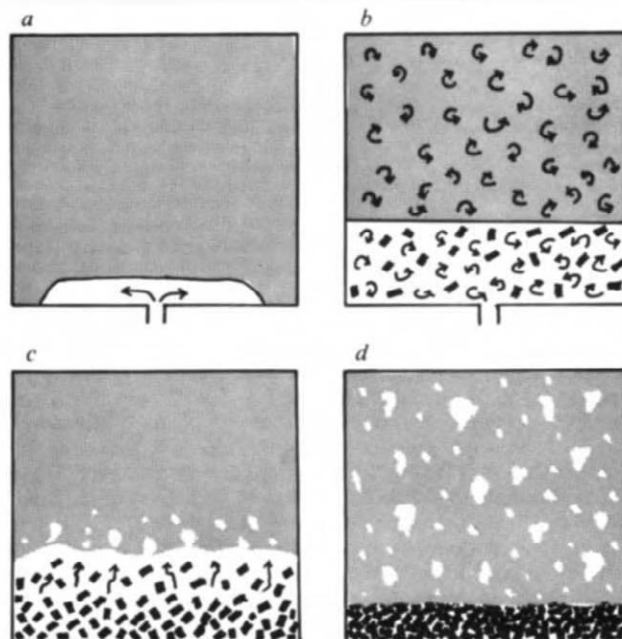


Fig. 2 Schematic model for the behaviour of a new influx of picritic melt into a MOR magma chamber (not to scale): a, intrusion of hot, dense picrite into reservoir of fractionating basalt; b, stable stratification into two layers which equilibrate thermally by turbulent convection with growth of olivine in lower layer; c, convection dies in lower layer at the end of cooling history allowing for olivine settling and separation of low density interstitial basaltic melt; d, Cumulate olivine layer formed at base of chamber as low density interstitial melt mixes convectively with heated upper layer.

a period of a few years at most. During its cooling to thermal equilibrium with the upper layer, the picrite will be highly turbulent and olivines precipitated from the liquid will remain in suspension until a late stage when the temperatures of the two layers are nearly equal. Provided that the upper layer is volumetrically large, the lower layer will transform into a mixture of suspended olivine in a basalt liquid. This basaltic liquid will be more primitive than the upper layer which has increased slightly in temperature. In many of our calculations, the residual liquid composition in the lower layer is estimated to occur near to the density minimum shown in Fig. 1. Note that the lower layer will in fact increase in bulk density as it crystallizes, as long as the olivines are kept in suspension. For example, the bulk density of liquid A plus 20% olivines in suspension is 2.82 g cm^{-3} . The dense lower layer remains in existence as long as the convection is sufficiently vigorous to suspend the olivine crystals.

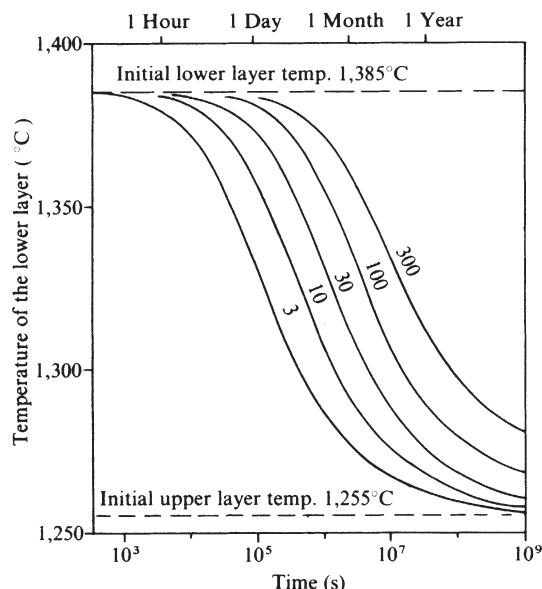


Fig. 3 Temperature of the lower layer as a function of time for various thicknesses (h) in metres of the lower layer for an upper layer 4 km thick.

Once convection has died down in the closing stages of cooling, theory¹⁶ predicts a concentration gradient of olivines will be generated in the lower layer when the free-fall velocity and convective velocities become comparable (Fig. 2c). Only a slight concentration gradient is required to nullify completely the density instability caused by the slight temperature gradient at this late stage, leading to a transition to stable stratification of the lower layer. We predict that the layer will pass rapidly to a stage of *en masse* sedimentation of suspended olivine. A substantial amount of basaltic liquid will segregate as the olivines sediment (Fig. 2c). For example, if a picrite layer, 100 m thick, contained 18% MgO at $1,400^\circ\text{C}$ and the upper layer, 2,000 m thick, contained basalt with MgO = 9% at $1,250^\circ\text{C}$, the lower layer would evolve to a suspension of 20% olivines in a basalt liquid at an equilibrium temperature of $1,264^\circ\text{C}$ and 10% MgO. Sedimentation of the olivine to a dense close-packed mode (60% olivine) would liberate ~80% of the remaining basaltic liquid, generating a cumulate layer 33 m thick (Fig. 2d). The olivines would be relatively uniform in composition as they have grown in conditions approximating equilibrium crystallization in the turbulent layer. Such features are found in the homogeneous olivine cumulates observed at the base of ophiolites. Further adcumulus growth (as is common in ultramafic cumulates) would drive out further basaltic liquid component.

Thermal equilibration between the two layers (Fig. 2) would require that the upper layer has increased in temperature. In many cases the upper layer would be more evolved and denser

than the basalt liquid derived from the lower layer as illustrated in the example calculation above. We conclude that the basalt liquid squeezed out during olivine sedimentation in the lower layer will be buoyant in the upper layer and will mix with it convectively (Fig. 2d). In this way the compositions of basalts which can erupt at the surface are restricted in composition as they periodically become mixed with basalt close to the density minimum.

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Volcanic rocks cored on Hess Rise, western Pacific Ocean

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Large aseismic rises and plateaus in the western Pacific include the Ontong-Java Plateau, Magellan Rise, Shatsky Rise, Mid-Pacific Mountains, and Hess Rise. These are relatively old features that rise above surrounding sea floors as bathymetric highs. Thick sequences of carbonate sediments overlie, what are believed to be, Upper Jurassic and Lower Cretaceous volcanic pedestals. We discuss here petrological and tectonic implications of data from volcanic rocks cored on Hess Rise. The data suggest that Hess Rise originated at a spreading centre in the late early Cretaceous (Aptian–Albian stages). Subsequent off-ridge volcanism in the late Albian–early Cenomanian stages built a large archipelago of oceanic islands and seamounts composed, at least in part, of alkalic rocks. The volcanic platform subsided during its northward passage through the mid-Cretaceous equatorial zone. Faulting and uplift, and possibly volcanism, occurred in the latest Cretaceous (Campanian–Maastrichtian stages). Since then, Hess Rise continued its northward movement and subsidence. Volcanic rocks from holes drilled on Hess Rise during IPOD Leg 62 (Fig. 1) are briefly described here and we relate the petrological data to the origin and evolution of that rise. These are the first volcanic rocks reported from Hess Rise.

Hess Rise is a triangular-shaped bathymetric high that is bounded on the west by the Emperor Seamount chain, on the north-east by the Emperor Trough, and on the south by the Mendocino Fracture Zone. A clear sequence of magnetic anomalies has not been discovered on Hess Rise, but it is believed to lie entirely within the Cretaceous magnetic quiet

zone and thereby is younger than early Aptian (~111 Myr old) in age. Our drilling results¹ and previous plate reconstructions^{2,3} indicate that Hess Rise formed in Aptian or early Albian time south of the early Cretaceous palaeoequator and moved progressively northward as part of the Pacific Plate.

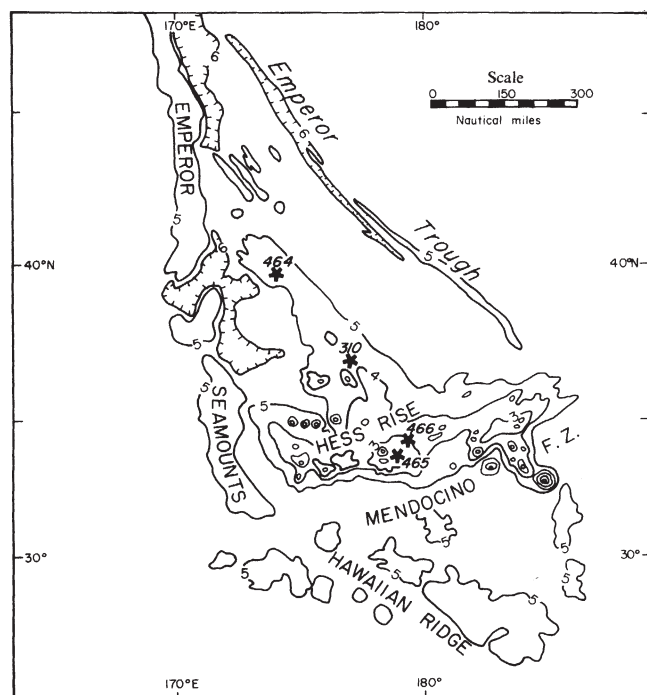


Fig. 1 Bathymetric chart of Hess Rise showing sites drilled and the major bathymetric features. Site 465 cores penetrated trachyte beneath upper Albian limestone and Site 466 cores contained alkali basalt clasts in Upper Cretaceous chalk. Site 464 cores penetrated altered tholeiitic basalt beneath lower Albian limestone. Site 310, drilled on DSDP Leg 32, did not penetrate igneous rocks. Contour intervals are in kilometres.

We recovered 16 cm of highly altered tholeiitic basalt from beneath Lower Albian (uppermost Lower Cretaceous) sediments at Site 464 on northern Hess Rise, 24 m of altered trachyte from beneath Upper Albian sediments at Site 465 on southern Hess Rise, and alkali basalt clasts in Upper Cretaceous (Campanian–Maastrichtian) sediments at Site 466 on southern Hess Rise. Petrological interpretations are based on the petrographical data from 68 thin sections, X-ray diffraction analyses of altered phases, whole-rock chemical analyses of 18 trachyte samples from Site 465 and three basalt samples from Site 464, and microprobe analyses of feldspar phenocrysts from Site 465 trachytes and clinopyroxene phenocrysts from Site 466 basalt clasts. The X-ray fluorescence and instrumental neutron activation analysis data for rocks from Sites 464 and 465 are given in Tables 1 and 2. Clinopyroxene data determined by microprobe analyses of basalt clasts from Site 466 are given in Table 3. Figures 2 and 3 are plots of rare earth element (REE) data.

The basalt from Site 464 is deeply altered as indicated by the high total water contents (7.12–8.05%), high K₂O contents, and abundant smectite minerals. The most convincing evidence that these are tholeiitic basalt, characteristic of mid-ocean ridge basalt (MORB), rather than alkalic basalt is the REE pattern (Fig. 2). The pattern is relatively flat at about 15 times the chondrite abundance and corresponds to a transitional type MORB⁴. In contrast, alkalic basalts show considerable light REE enrichment relative to heavy REE. Studies on the alteration of tholeiitic basalt by seawater^{5–7} show that basalt increases in the amounts of H₂O⁺, total Fe, K, and Ti and decreases in the abundances of Ca, Mg, and Si which are consistent with our results.

Table 1 Compositional ranges and averages of major and minor element oxides as determined by X-ray fluorescence, instrumental neutron activation analysis, and wet chemical techniques

Oxide	Trachyte (Site 465)		Basalt (Site 464)	
	Range	Average	Range	Average
SiO ₂	58.07–60.81	59.66	45.58–46.75	46.16
TiO ₂	0.99–1.07	1.03	1.72–1.75	1.74
Al ₂ O ₃	18.40–19.08	18.78	15.69–16.14	15.92
Fe ₂ O ₃ *	2.37–3.59	2.97	6.27–6.49	6.38
FeO	0.14–1.00	0.56	6.46–7.16	6.81
MgO	0.31–2.08	1.03	7.82–7.89	7.85
CaO	1.94–3.40	2.31	3.91–4.72	4.32
Na ₂ O	4.68–5.46	5.12	3.08–3.38	3.23
K ₂ O	2.89–6.40	4.80	0.81–1.68	1.25
P ₂ O ₅	0.28–0.46	0.36	0.09–0.10	0.09
MnO	0.015–0.060	0.034	0.25–0.29	0.27
H ₂ O ⁺	0.60–2.68	1.56	2.74–2.79	2.76
H ₂ O ⁻	0.64–3.98	1.84	4.33–5.31	4.82
CO ₂	0.21–1.73	0.47	—	—

* Fe₂O₃ is total iron for Site 465 analyses only.

Site 465 data (N = 18) are from southern Hess Rise trachyte and Site 464 data (N = 2) are from northern Hess Rise basalt.

Subaerial eruption of trachyte flows at Site 465 is suggested by large (>5 mm diameter) vesicles, red iron oxide staining in some flow units, and the absence of glassy flow margins. The trachytic textures are defined by flow-aligned feldspar laths and microlites in smectite-replaced glassy groundmasses. Plagioclase feldspar has a compositional range of An₂₈ to An₄₃ as determined by electron microprobe. Some plagioclase is completely replaced by potassium feldspar, possibly during a late-stage magmatic event.

Table 2 Trace element ranges and averages as determined by X-ray fluorescence and instrumental neutron activation analysis

Element	Trachyte (Site 465) (p.p.m.)		Basalt (Site 464) (p.p.m.)	
	Range	Average	Range	Average
La	75.1–89.1	82.5	4.8–5.1	5.0
Ce	156–187	171	12.8–13.6	13.2
Sm	8.9–11.7	10.5	3.1–3.5	3.2
Eu	2.9–3.2	3.0	1.17–1.25	1.20
Tb	1.0–1.6	1.3	0.66–0.69	0.68
Yb	1.8–4.4	2.9	2.37–2.96	2.27
Lu	0.3–0.7	0.5	0.32–0.42	0.36
Rb	15.3–66.5	33.6	—	—
Ba	596–688	645	—	—
Th	10.2–11.0	10.6	0.23–0.33	0.27
Hf	13.9–18.9	16.9	3.16–3.17	3.16
Ta	8.4–11.1	9.6	0.26–0.28	0.27
Zr	474–829	715	111–112	111
Sr	251–397	311	158–178	167
Co	8.5–22.6	16.2	38.6–50.3	45.3
Sc	2.1–3.2	2.7	55.0–55.8	55.3

For trachyte, N = 18; for basalt, N = 3.

Chemical data for trachyte samples from Site 465 (Tables 1 and 2) show that the oxides and elements have small ranges of values. Most data trends are consistent with either differentiation effects or high temperature alteration. For example, MgO decreases as K₂O increases and K₂O increases with SiO₂ as expected for differentiation. However, both K₂O and SiO₂ decrease as H₂O⁺ increases and MgO increases as H₂O⁺ increases. These data are consistent with effects caused by high-temperature alteration^{8,9}. Rare earth data for the trachyte samples are plotted in Fig. 3. The REE patterns are consistent with patterns for differentiated alkalic rocks. The heavy REE, particularly Lu, also decreases with increasing H₂O⁺. Despite their alteration, the trachytes from Hess Rise are chemically similar to trachytes from oceanic islands, particularly the Tristan da Cunha group¹⁰, and are also similar to end members of differentiation trends discovered in rocks from other oceanic rises and plateaus^{11–14}.

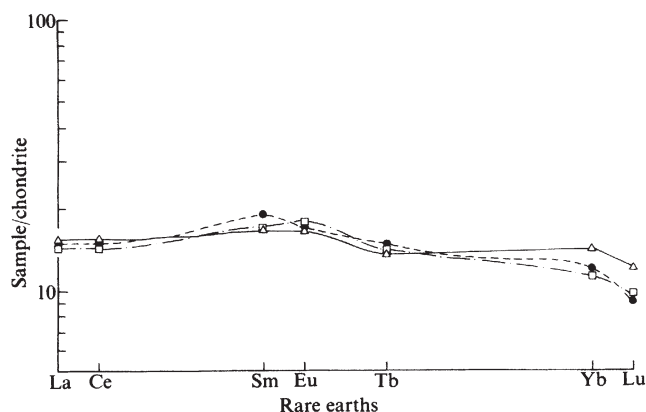


Fig. 2 Normalized plot of rare earth element abundance in basalt of Site 464. This plot is typical of tholeiitic basalt. Δ , 464-34-CC, 11 cm; $\bullet$, 464-34-CC, 19 cm; $\square$, 464-34-CC, 22 cm.

Alkali basalt clasts were recovered from Upper Cretaceous (Campanian–Maastrichtian stages) sediments at Site 466. The rocks have phenocrysts of olivine (completely replaced), clinopyroxene, and plagioclase enclosed in altered glassy groundmasses. The alkalic nature of the rocks is suggested by the high calcium contents of clinopyroxene phenocrysts (Table 3). Plots of the molecular percentages En–Fs–Wo place the pyroxenes in the salite and high-calcium augite fields. Stratigraphic and petrological data from the clasts indicate that erosion of alkalic basalt islands occurred at least 25 Myr after the main volcanic pedestal had formed. It is apparent that some uplift and faulting took place in the late Cretaceous, but the reason for the tectonic activity is not known.

The age, as determined by overlying and associated biogenic sediment, and petrology of the volcanic rocks have important tectonic and petrological implications. Combined with our interpretations based on shipboard data¹ and recognition of plate-associated lateral and vertical movements^{2,3}, we can present some preliminary conclusions with regards to the geological history of Hess Rise.

Table 3 Clinopyroxene chemistry of two alkali basalt clasts in Upper Cretaceous sediment, Site 466, southern Hess Rise.

Oxide	1	2	3	4	5
SiO ₂	50.57	51.09	48.17	49.97	48.16
TiO ₂	0.89	0.91	1.41	0.88	1.25
Al ₂ O ₃	3.14	3.39	5.76	4.32	5.50
Cr ₂ O ₃	0.22	0.29	0.22	0.23	0.36
FeO	7.29	7.47	8.20	7.11	7.59
MnO	0.13	0.13	0.11	0.11	0.13
MgO	14.50	14.45	12.98	13.66	13.50
CaO	22.67	21.73	22.07	22.40	22.16
Na ₂ O	0.45	0.53	0.74	0.61	0.64
Total	99.86	99.99	99.66	99.29	99.29
Molecular percentage					
Wo	46.7	45.6	47.4	47.7	47.3
En	41.6	42.2	38.8	40.5	40.1
Fs	11.7	12.2	13.7	11.8	12.6

Column 1, Small phenocryst, Sample 1; 2, Phenocryst core, Sample 2; 3, Phenocryst edge, Sample 2; 4, Pyroxene lath, Sample 2; 5, Small phenocryst, Sample 2. Analyses were done by Florence Lee-Wong, US Geological Survey.

Hess Rise, at least in part, is a subsided oceanic volcanic archipelago that was buried by younger pelagic sediments. Active volcanic activity probably ended before late Albian time, although the presence of alkali basalt clasts in the Upper Cretaceous strata at Site 466 may indicate later volcanism. The age difference between oldest sediment cored at the northern (464) and southern (465) sites (early Albian and late Albian–

Cenomanian respectively) is consistent with passage of the rise over a mantle hot spot, but this hypothesis needs additional testing.

The volcanic rocks we recovered from Hess Rise represent only the uppermost part of the pedestal; therefore, we do not know the nature of the underlying volcanic succession. Judging from studies of older parts of deeply eroded oceanic islands and seamounts, of rocks erupted from mid-ocean ridges, and of our data from sites on Hess Rise, we presume that the oldest volcanic rocks are tholeiitic basalt that were extruded at a

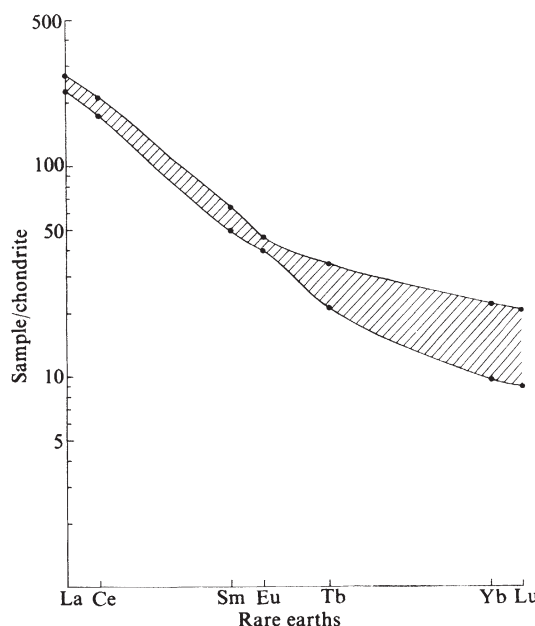


Fig. 3 Normalized plot of the total range of rare earth element abundance in Site 465 trachyte.

spreading centre in Aptian or early Albian time. Younger intraplate, or off-ridge, volcanism commenced after the sea floor had migrated from the ridge axis and subsequently built a large archipelago. After volcanic activity ceased, the archipelago continued moving northward. Most of the archipelago had subsided beneath sea level by the time it crossed the biological high-productivity zone of the mid-Cretaceous equator¹. Tectonic activity, probably mostly block faulting, raised parts of the old archipelago above sea level where subaerial erosion provided clasts that became incorporated in the Upper Cretaceous strata at Site 466. Synchronous volcanic activity may have occurred. Hess Rise continued to subside during the Cenozoic as it moved northward and is now a prominent bathymetric high in the western Pacific.

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Unique amino acid composition of Red Sea brine

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The lack of evidence from terrestrial environments supporting the chemical evolution model has resulted in scientists looking elsewhere in the Solar System and in space for evidence. We have suggested that the Atlantis II Deep brine (21°22'N by 38°05'E) located in a submarine rift at the bottom of the Red Sea is a promising environment possibly containing terrestrial evidence for chemical evolution¹. We have also reported finding a thiocyanate concentration of 2.4×10^{-5} M in the Atlantis II Deep brine². We report here the discovery of an unusually large concentration of glycine in the Atlantis II Deep brine and virtually no other dissolved free amino acids. We further suggest four possible explanations for the unique amino acid composition of Red Sea brine. Our methods of location, sampling and storage of the brines have been discussed elsewhere².

Several investigators have analysed seawater and other saline solutions for their dissolved free amino acid composition³⁻⁶. Because in deep ocean waters the dissolved free amino acid concentration is extremely low⁶, great care must be used in the sampling, storing and analysing the samples. Special precautions in our work included the following measures. All water used was double distilled in glassware. All glassware was acid (nitric acid/sulphuric acid, 1:2 by volume) washed and rinsed repeatedly with double distilled water. Before use, all inorganic compounds were heated at 450 °C for over 12 h to remove organic contaminants. Preparation of the reagents for chromatography entailed the following steps. Ammonium hydroxide was prepared by bubbling ammonia gas through acid-washed tubing, a water trap to remove any amino acids, and finally into water to produce 3 M NH_4OH . Hydrochloric acid reagent was made by two successive azeotropic distillations producing 6 M HCl.

Separation of the amino acids from the brine was accomplished by a liquid-exchange chromatography procedure using a copper-Chelex 100 (BIO-RAD) resin column and an ammonium-Chelex 100 column^{4,6}. For column preparation and separation, these steps were followed in the order indicated: (1) 25 ml of 3 M NH_4OH were passed through 10 ml of Cu^{2+} -Chelex 100; (2) the column was rinsed with water until pH 9.8 was reached; (3) 0.3 ml of 1 M NaOH was added to 10 ml of brine. The solution was brought up to 100 ml with water and poured onto the column; (4) the column was flushed with water until Cl^- free as measured visually using AgNO_3 solution; (5) amino acids were eluted with 25 ml of 1.5 M NH_4OH ; (6) 20 ml of 1.5 M NH_4OH were passed through 5 ml of NH_4^+ -Chelex 100; (7) the eluant amino acid solution was passed through the NH_4^+ -Chelex 100 column to separate Cu^{2+} from the eluant; (8) the column was chased with 5 ml 1.5 M NH_4OH ; (9) the sample was lyophilized. 2 ml of double glass distilled water was added, the solution was centrifuged, the supernatant removed and re-lyophilized.

Analysis was performed using a Beckman 121 MB amino acid analyser equipped with a model 126 data system. Samples were applied to a 0.28×20 cm column with Beckman AA-10 resin using a standard sodium citrate program (Beckman) with ninhydrin detection in a single column system.

Red Sea brine samples and several kinds of controls were all analysed for amino acids using identical procedures. The controls included many duplicates of 5 M NaCl synthetic brine, non-brine Red Sea water from a depth of 500 m, a solution of known amounts of amino acids, known contaminated solutions, the ammonium hydroxide reagent, the double glass distilled water and the hydrochloric acid used in preparing the resins. The synthetic brine and ammonium hydroxide revealed a glycine contamination level of <50 pmol per 10 ml. The water and acid were below the detection levels of the system. Recovery of known amino acids averaged ~50%. Known contaminated solutions showed a wide variety of amino acids.

The Atlantis II Deep brine samples yielded a mean glycine concentration of $1,090 \text{ nmol l}^{-1}$ based on 11 analyses. About 10 nmol l^{-1} or less of serine was also detected. No other dissolved free amino acids were detected. The non-brine Red Sea sample showed a glycine concentration of $\sim 122 \text{ nmol l}^{-1}$.

Previous reports of amino acids in deep ocean water suggest that a wide variety of dissolved free amino acids are present in most cases, but the total concentration levels are always below 100 nmol l^{-1} (ref. 6). Surface ocean water samples are reported to contain $\sim 1,000 \text{ nmol l}^{-1}$ with glycine comprising $\sim 200 \text{ nmol l}^{-1}$ (ref. 5).

How can the presence of high amounts of glycine and virtual absence of other amino acids be explained? As the glycine concentration is higher than that found in highly productive surface coastal waters, even though the brine is apparently sterile, an additional source must be sought. We suggest that one plausible source is an abiotic origin involving a Strecker synthesis of amino acids from aldehydes and cyanide. Because the methane content is known to be high, and formaldehyde is easily derived from methane, one can then explain the high glycine concentration. Why glycine is virtually the only amino acid found is unclear. Some investigators have invoked the properties of clays to explain unique amino acid distributions^{8,9}. As relatively large volumes of samples are needed to check carbon isotope ratios, we have not yet determined the $^{12}\text{C}/^{13}\text{C}$ ratio which could shed light on the origin of the glycine. There are several other plausible explanations for our results. The most obvious is that there is biological contamination of the brines and/or our procedures. The Atlantis II brine has been reported sterile⁷. As the amino acid distribution of our control brine indicates no contamination and bacteriological assays done on water from the same sampler were negative (R. Cuhel, personal communication), we consider this explanation unlikely. Another explanation might involve chemical contamination of our sample. Glycine has been reported to bleed from or through Chelex 100⁶. If this were the explanation for our data, why were there such repeatable small amounts of glycine in the synthetic control brine? A third alternative is that the abundant bacteria above the Atlantis II brine decompose organisms and the amino acids leach into the brine where they accumulate. Perhaps there is some chemical compound in the brine that selectively removes all dissolved amino acids except glycine from the brine, or alters other amino acids to form glycine. However, there is no evidence to support this.

If amino acids are synthesized in geothermal systems, this might provide the nutrient base for some of the unique, highly productive regions surrounding deep-sea springs.

We have demonstrated the value of further exploration of Red Sea hot brines and especially the Atlantis II Deep brine from the perspective of chemical evolution. This should be done as soon as proposals have been made to mine commercially the rich heavy metal deposits in the sediment beneath the brines.¹⁰

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Size distribution of Amazon River bed sediment

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The first recorded observations of bed material of the Amazon River were made in 1843 by Lt William Lewis Herndon of the US Navy, when he travelled the river from its headwaters to its mouth, sounding its depths, and noting the nature of particles caught in a heavy grease smeared to the bottom of his sounding weight¹. He reported the bed material of the river to be mostly sand and fine gravel. Oltman and Ames took samples at a few locations in 1963 and 1964, and reported the bed material at Óbidos, Brazil, to be fine sands, with median diameters ranging from 0.15 to 0.25 mm (ref. 2). We present here a summary of particle-size analyses of samples of streambed material collected from the Amazon River and its major tributaries along a reach of the river from Iquitos in Peru, ~3,500 km above Macapá, Brazil, to a point 220 km above Macapá³.

The field work was done aboard RV Alpha Helix. Samples were collected with pipe dredges³, or with a US BM-54 bed material sampler⁴, dried aboard ship, and transported to the US Geological Survey laboratories in Denver, Colorado, for particle-size analysis by sieving, or by pipette-visual accumulation tube methods⁵.

Herndon's observations were remarkably accurate; the bed of the Amazon is mostly fine sand. Many of our samples contained small percentages of fine gravel; these were especially prevalent at locations where there were local outcrops in the abutting terraces, or where conditions favoured hydraulic sorting; for example, on the outsides of bends, downstream from

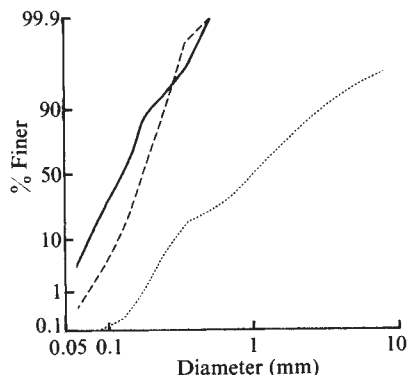


Fig. 1 Hydraulic sorting at the confluence of the Rio Negro and Rio Solimões results in the local deposits of gravel. The water depth was 54 m. Curves are coded as follows: confluence of the Rio Negro and Rio Solimões; ----, Rio Amazonas, 74 km downstream of the confluence; —, Rio Solimões, 131 km upstream of the confluence.

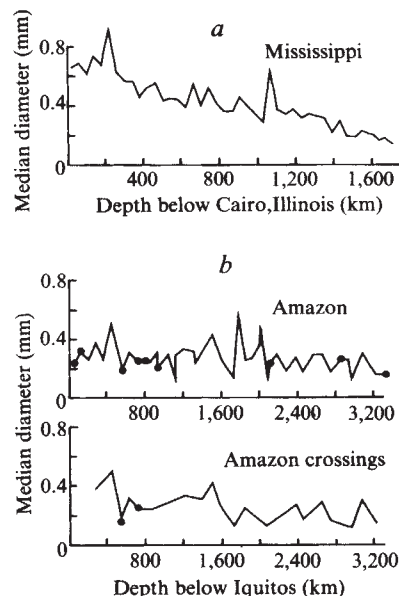


Fig. 2 Variation with distance of median particle size: *a*, for the Mississippi River; *b*, for the Amazon River. Median sizes shown for Mississippi are averages of sands (median diameters 0.06–2.00 mm) for 40-km reaches of river. Median sizes shown for Amazon are mostly individual samples; ●, average values where two or more samples were collected in a cross-section.

confluences with some of the major tributaries, where there are zones of high shearing both in the flow and at the bed with exceptionally high turbulence, or in very narrow sections with high velocities. Figure 1 shows a comparison of particle-size distributions of material collected in a deep hole just below the confluence of the Rio Negro and the Rio Solimões, and of material collected 131 km upstream and 74 km downstream of the confluence. The striking differences in size distribution are apparently due to hydraulic sorting at the confluence in combination with local outcrops of fine gravel in the Alter do Chão formation.

In most alluvial streams, bed material becomes finer and more uniform in a downstream direction. Figure 2*a*, for example, shows the trend of decreasing particle size for bed material of the Mississippi River along a 1,600-km reach below Cairo, Illinois⁶. The mean particle diameter along the reach is reduced from ~0.7 to 0.2 mm.

No such striking reduction in particle size was found for the Amazon River between Iquitos and Belem. A plot of the median diameters of bed materials from our 1977 samples against distance downstream from Iquitos, Fig. 2*b*, shows no significant trend. To eliminate the effects of local hydraulic sorting, we systematically collected some of our samples on the crossings between bends. When these data are plotted by themselves (Fig. 2*b*), a slight trend towards decreasing particle size is apparent.

At several cross-sections where we measured the suspended-sediment discharge^{7,8}, we collected five or more samples of the bed at equally spaced intervals across the sections. The average curves (Fig. 3) show no appreciable differences in particle-size distributions. Cross-channel variation in particle size at several cross-sections was at least as great as downstream variations encountered along the entire 3,300-km reach.

A sorting coefficient, σ , was computed for each sample:

$$\sigma = 1/2 \left(\frac{d_{50}}{d_{16}} + \frac{d_{84}}{d_{50}} \right) \quad (1)$$

where the subscript represents per cent finer. For example, d_{50} is the particle size for which 50% by weight is finer. Values of σ ranged from 1.18 to 2.59, with 70% of the values falling between 1.20 and 1.40. The highest value, 2.59, was for the

sample taken at the confluence of the Rio Negro and Rio Solimões, the size distribution of which is shown in Fig. 1. When values of σ were plotted against distance, no significant trend was found. The bed material does not tend to become more uniform in the downstream direction, as it does in most other alluvial streams.

Although the median size and sorting coefficient of the sands on the bed of the Amazon mainstem are essentially constant in the 3,300-km reach we sampled, the composition of the sands in the same reach changes significantly. To the unaided eye, the sands collected near Iquitos appear darker than those collected farther down the river, because of a larger proportion of dark mineral grains and rock fragments. Heavy-mineral suites also differ.

Landim *et al.*⁸ report that sands in the first 1,300 km or so downstream from Iquitos are rich in unstable minerals, such as hypersthene, augite and amphibole; these indicate a predominantly Andean source. Sands in the lower 2,000 km contain fewer unstable minerals and higher proportions of zircon and tourmaline. This change in mineral composition is unlikely to represent only the progressive decomposition of unstable Andean minerals during transport or during temporary storage in alluvial deposits, because such decomposition would

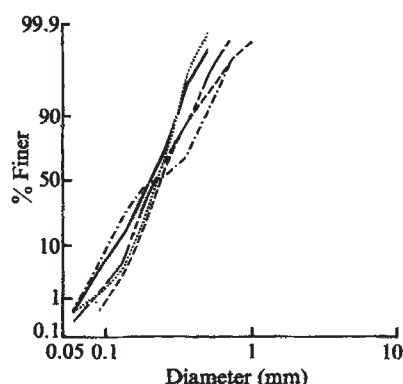


Fig. 3 Average particle-size distribution curves for cross-sections where five or more samples were collected. The curves are coded as follows: —, Rio Marañon, 25 km below Iquitos; ---, Rio Solimões at São Paulo de Olivença, 757 km below Iquitos; ···, Rio Solimões near Santo Antonio do Iça, 909 km below Iquitos; - · - ·, Rio Solimões at Manacapuru, 2,086 km below Iquitos; - - - -, Rio Amazonas at Óbidos, 2,827 km below Iquitos.

be accompanied by attrition and a reduction in particle size along the channel. We hypothesize that the change in composition represents a combination of the decomposition of the Andean minerals and a dilution of that mainstem material by sands brought in by tributaries from the Precambrian shield areas north and south of the Amazon. Thus, any reduction in size of material from the Andean sources by attrition along the mainstem below Iquitos is offset by the addition of material from tributaries draining the Precambrian shield areas.

A family of regression equations describing the density distribution of dispersing organisms

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I have previously shown¹ that some of the published regression equations for describing the decline of density of insects with distance from a centre of dispersal were each special cases of a more general equation:

$$N = \exp(a + bx^c) \quad (1)$$

where N is density at a distance x from a centre of dispersal and a , b , and c are parameters. Equation (1) is a good description of the dispersal of most insects so far investigated¹. To describe any single set of data, a simpler expression can often be found to fit, but this approach will lead to as many theories of migration as there are equations. To describe all sets of data by a single generalized model may require greater complexity than equation (1), and more parameters. I present here such a generalization, which may lead to a unified theory.

The decline in density with distance is characteristically concave, being steep near the origin, and having a long tail, but more rarely the relationship is convex in form (for example, *Meligethes aeneus*^{1,2}); permitting the exponent in equation (1) to vary enables both forms to be fitted by the same equation. But a problem with equation (1) is that as c approaches zero a singularity occurs which is difficult to interpret, leading as it does to $N = \exp(a + b) = a$ constant. When c approaches zero it is appropriate to adopt the logarithmic equation,

$$N = \exp(a + b \ln x). \quad (2)$$

The relationship between c and the residual variance after fitting equation (1) is a continuous function, except in the region of $c \sim 0$. As c approaches zero, equation (2) has been found to be a good fit for one set of data (*Glossina palpalis* and *G. tachinoides*^{1,3}) of the eight previously examined, and the residual variance was the value which preserved the relationship between c and residual variance after fitting equation (1).

Early models used to describe dispersal have usually been derived from the normal distribution and are thus closely related to the diffusion equations of physics. In these models it is assumed that particles, or in this case, insects, separate at a constant velocity known as the coefficient of diffusion D , and for a given period. When applied to living organisms this assumes that their behaviour is fixed and is unaffected by the presence of others: an assumption with little justification⁴.

The regression equation related to the diffusion equation is the so-called 'half-normal',

$$N = \exp(a + bx^2) \quad (3)$$

in which b is inversely proportional to the coefficient D . By assuming that D is proportional to x^{2-c} , equation (1) is obtained. With D proportional to distance x , the rate of diffusion must be inversely proportional to density, a relationship which can be interpreted biologically. A large number of animals released at a point are likely to interact with one another, changing their behaviour in a density-dependent fashion; high densities experienced during rearing or collection may lead to longer periods of activity. They also increase contacts between individuals which may, in turn, reduce the initial speed of movement away from the release

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Table 1 Parameter estimates

Species	Ref.	a	b	c	d	d.f.	r.m.s.	F-ratio
<i>Drosophila pseudoobscura</i>	6	3.48	-0.03	0.83	-0.18	16	0.9376	356.41
<i>Musca domestica</i>	12	-4.22	51.59	-0.63	0.49	23	0.4483	31.95
<i>Phormia regina</i>	13	1.95	8×10^{-5}	-13.81	-0.61	15	4.4483	78.24
<i>Lucilia sericata</i>	14	2.90	-1.60	0.43	0.16	10	0.7456	20.93
<i>Glossina palpalis</i> + <i>tachinoides</i> *	3	11.16	-3.72	1×10^{-3}	-0.99	14	1.2205	32.38
<i>Erioischia brassica</i>	15	3.80	-0.62	0.65	0.29	13	1.3192	96.86
<i>Scolytus multistriatus</i>	16	5.78	-2×10^{-12}	3.98	-0.59	6	9.2005	27.93
<i>Meligethes aeneus</i>	2	-17.94	1×10^{-4}	1.40	3.17	18	0.1360	50.56
<i>Danaus plexippus</i>	17	8.44	-4.25	0.14	0.64	4	4.9775	124.24
<i>Larus argentatus</i>	18	7.98	-1×10^{-5}	1.76	-0.68	4	9.7525	487.21
<i>Larus novaehollandiae</i>	19	2.69	-8×10^{-3}	1.99	-0.19	15	0.1257	673.50
<i>Parus major</i>	20	3.99	-2.40	0.47	2.00	13	0.4232	109.78
<i>Turdus merula</i>	21	7.80	-3.36	0.15	5×10^{-3}	4	6.7125	2128.72
<i>Homo sapiens</i>	22	3.17	-0.01	0.03	-0.93	12	2.8050	353.80
Peorian loess	10	2.66	-0.05	9×10^{-4}	-0.43	12	1.3775	125.71

Equation (5) fitted to dispersal data of 14 species, and also that of the deposition of Peorian loess in central North America. (Significance levels all less than 0.001.) The data are all of average numbers obtained at a distance from a point source except for the tsetse* data which are from a line source, and standardized for density (see ref. 23). The *Drosophila* data are of the combined data of Dobzhansky and Wright¹ as given in Wallace²⁴. d.f. = degrees of freedom, r.m.s. = residual mean square.

point, thus decreasing the diffusion coefficient. As the number of contacts increases with the number released and a very large number of dispersing insects is needed to get sufficient recaptures for analysis, such experiments are likely to decrease the diffusion coefficient in the early stages of dispersal. Once an insect has left the central concentration, its diffusion coefficient may rise to some characteristic level determined by its earlier environmental history; the amount of interference required for a given effect is also likely to be a species characteristic.

This complex behaviour makes the diffusion process envisaged by Pearson⁵ and Dobzhansky and Wright⁶ seem unlikely; yet the prediction that the variance of distance flown should be linearly related to the time spent flying was apparently upheld by Dobzhansky and Wright's experiment⁶. Their discovery that the diffusion coefficient was constant only for a given experiment did not deter their faith in the model, but the fact that the coefficient varied between experiments⁷ casts some doubt on the validity of the normal distribution as a basis for the model. With the potential for such varied behaviours in different species, it seems essential to increase the numbers of model parameters. While this may present problems in statistical estimation, it does make possible a rational search for behavioural interpretation that is not possible with the normal model⁸.

The distribution from which equation (1) presents a form appropriate for regression analysis is a generalization of the Γ distribution (when $\gamma=0$), and which also includes the Weibull distribution ($\alpha=0$), and the half-normal ($a=\frac{1}{2}$, $c=2$, $\gamma=0$), and which has the probability density function⁹

$$p(x) = \frac{c(x-\gamma)^{c\alpha-1}}{\beta^{c\alpha}\Gamma(\alpha)} \exp\left(-\left[\frac{x-\gamma}{\beta}\right]^c\right). \quad (4)$$

Equations (1) and (4) are related by ($\gamma=0$), ($\beta^{-c} = -b$), ($\alpha = 1/c$), and ($c/\beta\Gamma(1/c) \equiv \exp(a)$). If the condition that ($\alpha=1/c$) is relaxed, a second term in x is obtained, and defining equivalent parameters gives,

$$N = \exp(a + bx^c + d \ln x) \quad (5)$$

where ($\gamma=0$), ($\beta^{-c} = -b$), ($c\alpha = d+1$), and ($c/\beta^{c\alpha}\Gamma(\alpha) \equiv \exp(a)$). Equation (5) has equations (1) and (2) as special cases; equation (1) is obtained by setting $d=0$ and equation (2) when either b or $c=0$. In view of this relationship, the $\ln x$ term of equation (5) clearly becomes dominant as b or c approaches

Table 2 Residual SS after fitting equation

Species	Ref.	(3)	(2)	(1)	(5)	F-ratio
<i>Drosophila pseudoobscura</i>	6	160.92	86.07	15.81*	15.00	0.8650 NS
<i>Musca domestica</i>	12	31.43	18.81	10.35*	10.31	0.0914 NS
<i>Phormia regina</i>	13	167.06	121.23	79.02*	69.49	2.0571 NS
<i>Lucilia sericata</i>	14	13.19	11.32	7.46*	7.46	0.0080 NS
<i>Glossina palpalis</i> + <i>tachinoides</i>	3	25.65	17.09*	17.09	17.09	0.0004 NS
<i>Erioischia brassica</i>	15	18.62	20.40	17.17*	17.15	0.0171 NS
<i>Scolytus multistriatus</i>	16	232.17	81.78	64.77*	55.20	1.0040 NS
<i>Meligethes aeneus</i>	2	6.98	10.76	6.20*	2.45	27.5809 †
<i>Danaus plexippus</i>	17	144.79	69.86	21.43*	19.91	0.3052 NS
<i>Larus argentatus</i>	18	1330.52	302.43	48.53*	39.01	0.9763 NS
<i>Larus novaehollandiae</i>	19	4.61	27.89	1.92*	1.89	0.2625 NS
<i>Parus major</i>	20	17.71	84.59	16.66*	5.50	26.3686 †
<i>Turdus merula</i>	21	892.94	112.70	26.86*	26.85	0.0021 NS
<i>Homo sapiens</i>	22	525.40	33.65*	34.40	33.65	0.0005 NS
Peorian loess	10	22.21	16.53*	16.56	16.53	0.0018 NS

Residual sums of squares after fitting the four regression equations to the 15 sets of data.

*The F-ratio is the comparison of equation (5) with the next best fitting equation indicated by an asterisk.

†Significance shows that the complete four-parameter equation is required for adequate description of the dispersal data ($P < 0.001$). As expected, equation (5) has the minimum RSS for all data, but not necessarily the lowest mean square residual.

NS indicates no significant improvement in fit by using equation (5); SS = sums of squares, RSS = residual sums of squares.

zero, and shows why $N = \exp(a + b \ln x)$ is an appropriate alternative equation preserving the symmetry between c and residual variance. Thus the description of Morris' data of tsetse dispersal³ is not qualitatively different from the other seven sets investigated in ref. 1.

Using literature data for several taxa, I attempted to determine the usefulness of these equations for description of animal dispersal from a point source. Table 1 shows the results of fitting equation (5) to data of 14 animal species and also, for comparison, one set of data of the aerial displacement by wind of grains of Peorian loess in central North America¹⁰. Table 1 shows that all the data fit the model well, and also that they can be separated into three types; two data sets have d comparatively large (*Meligethes* and *Parus*) suggesting that the fourth parameter has a large influence, and three sets in which c is small (*Glossina*, man and soil), supporting the logarithmic equation (2): of the remainder, approximately half have very small b but these coincide with large c .

To confirm that equations (1) and (2) are indeed special cases of equation (5) when b , c or d are small, all three equations were fitted to the data, with, for comparison, the 'half-normal' equation (3). Table 2 shows the residual sums of squares after fitting the four equations. The last column shows the F -ratio for improvement obtained by fitting equation (5) over the next best fitting equation, and equation (1) always fits better, usually significantly better than equation (3). Note that only man and the tsetse fly data conform to the same model as the soil. What features do man and tsetse have in common with air-blown soil? It has been suggested that all behaviour is the resultant of two opposing tendencies, to approach and to separate, and that the distributions we see of organisms in space are the sum of these two opposing behaviours¹¹. The distribution of Peorian loess is probably also the result of characteristics of the grains which tend to promote or inhibit displacement by wind, for example, surface texture and mass. I do not suggest that the forces promoting displacement in animals are the same as for wind-blown soil, but that there are analogous forces acting in both cases.

Whatever those forces are, the same functional relation connecting them to the specific behaviour of the dispersing particle (soil, spores and seeds as well as actively moving animals) should apply. The equation presented here is a good candidate for such a function, even though at this stage the parameters cannot be identified. However, it is likely that the segregation of the data sets into two groups described by equations (1) and (2) does reflect a real behavioural difference in movement. To determine what that behaviour might be will necessitate analysis of the parameters and this requires far more density-distance data than is available.

Cameral liquid transport and buoyancy control in chambered nautilus (*Nautilus macromphalus*)

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Studies of cephalopod buoyancy¹ show that transport of cameral liquid by the shallow water cuttlefish (*Sepia*) can be explained by a reduction of the liquid's osmotic pressure, followed by osmosis out of the chamber. This mechanism seems insufficient to explain chamber emptying in the chambered nautilus (*Nautilus macromphalus*), which is frequently found below 240 m, the depth beneath which simple osmotic emptying cannot function^{2,3}. At 240 m, the hydrostatic pressure forcing water into the chambers of the nautilus shell exceeds the maximum osmotic emptying pressure. Because deep water experiments were not feasible, it was our aim to determine whether nautilus could transport cameral liquid against osmotic pressure gradients, and to consider mechanisms that could account for transport against both osmotic and hydrostatic gradients. These possible mechanisms include enhanced siphuncular blood concentration (possibly caused by a countercurrent multiplier in the siphuncle⁴) which could 'draw' water from chambers below 240 m, and 'local osmosis'⁶, resulting from enhanced solute concentrations in siphuncular intercellular spaces. Physiological and ultrastructural evidence for the latter is presented here.

To expose the siphuncle to osmotic pressure differences tending to draw water into a chamber, we drilled a hole into a 20-ml chamber and removed any liquid. Then we added 5 ml of NaCl-enriched seawater (either 1.3 or 1.9 osmolal). To give the animals an 'incentive' to empty their chambers, we drilled a hole in an adjacent chamber and filled it with seawater, causing the animal to sink to the bottom of the aquarium (1 m deep).

After 10–15 days we measured volumes and osmolalities of liquid remaining in the experimental chambers. Of 13 animals, 12 transported some liquid out (the animal that did not was omitted). For nine animals with 1.3-osmolal solutions in their chambers, the rate was $159 \mu\text{ld}^{-1} \pm 80$ (95% confidence interval), and for three animals whose chambers contained 1.9 osmolal solutions, $218 \mu\text{ld}^{-1}$. The osmolality of heart blood of these and other animals was 1.062 (range 1.054–1.069, 17 animals); the osmolality of the seawater was 1.045. By the end of the experiment the osmotic pressure of the experimental cameral liquids had fallen to 1.262 and 1.770 osmolal, respectively, still well above the blood concentrations. Siphuncular blood samples were not obtained.

Thus the siphuncle transports liquid by some mechanism other than simple osmotic flow. This transport against osmotic gradients, as well as transport against hydrostatic gradients, such as would occur below 240 m, could be explained by local osmosis, which requires a cellular architecture of intercellular spaces or basolateral infoldings of the transporting cells⁷. According to Denton⁵, siphuncular cells are "packed with mitochondria" and the basal and lateral membranes of these cells are "folded to form canaliculi". This description recalls other transporting cells thought to use local osmosis⁸. Our observations of siphuncular cells (Fig. 1) are consistent with this description. Our micrograph is from a siphuncle that was emptying cameral liquid (1.7 osmolal) against the osmotic gradient at a rate of $310 \mu\text{ld}^{-1}$. The numerous mitochondria along the lateral infoldings (canaliculi) suggest that solute-coupled water transport occurs at these small infoldings rather than at the level of the large basal infoldings. Neither large basal infoldings nor canaliculi are present in siphuncles that

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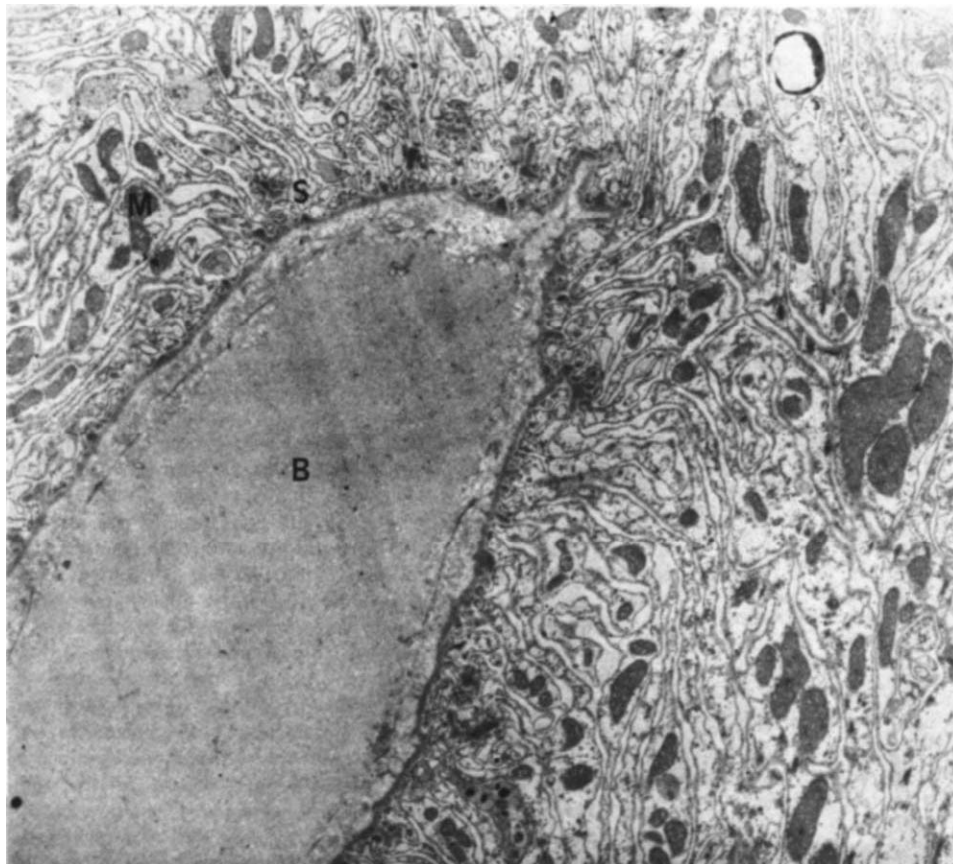


Fig. 1 Low power electron micrograph ($\times 5,000$) of a siphuncle cell. The large basal infolding (B) can be seen as well as the network of small channels (S) that lead into the large basal infolding. Numerous mitochondria (M) can be seen in the cytoplasm between the small channels.

have not yet begun to empty cameral liquid.

Nautilus adjusts its rate of emptying and consequent buoyancy development to meet its needs. When we did not flood incentive chambers and the animals were buoyant, as they become in the laboratory, emptying of hyperosmotic solutions was slow (Fig. 2). These animals increased their emptying rates when incentive chambers were flooded, to make the animals sink. Other animals that had been emptying rapidly (and had flooded incentive chambers) decreased their emptying rates when we emptied and plugged the incentive chambers to make the animals float.

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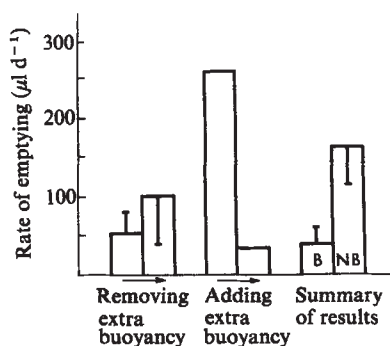


Fig. 2 Rates at which cameral liquid was emptied from experimental chambers of nautilus in different conditions. See text for explanation. The lines extending from the means are the 95% confidence intervals. The difference in emptying rates between all buoyant (B) and non-buoyant (NB) animals (last panel) is highly significant ($P < 0.01$, Student's t test).

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Ovipositing butterfly (*Pieris brassicae* L.) distinguishes between aqueous extracts of two strains of *Cannabis sativa* L. and THC and CBD

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The ovipositing female butterfly has proved a useful tool for separating closely related strains of various plant species. Using the large white (*Pieris brassicae*), we have been able to distinguish between the Turkish and Mexican strains of *Cannabis sativa*, and demonstrate that the butterfly is sufficiently sensitive to differentiate between purified THC (tetrahydrocannabinol) and CBD (cannabidiol) – two substances which taste and smell alike to the human observer.

Table 1 Oviposition on cabbage sprayed with aqueous extracts (soups) from leaves of *Cannabis sativa*

<i>Cannabis</i> strain and spray used on cabbage leaf	No. of trials	Egg batches (excluding singles)	No. of eggs laid	No. of times first choice of ovipositing females	Remarks
Cabbage extract v Tap water	4	35	482*	1	No significant difference
Boiled cabbage extract v Cabbage extract	2	ND	1,141	ND	The slight deterrent in extract seems to be dissipated by boiling away volatiles
Mexican extract v Tap water	18	8	135*	All tap water	Powerful deterrent present in Mexican strain
Mexican extract v Untreated cabbage leaf	10	3	132	1	Little if any difference between trials of treated or untreated leaves
Mexican extract, 2 leaves v Untreated cabbage, 1 leaf	3	52	1,493	9	
Mexican extract, 2 leaves v Untreated cabbage, 1 leaf	3	0	0	All cabbage	Two leaves did not increase laying on <i>Cannabis</i> sprayed cabbage
Untreated cabbage, 1 leaf		31	1,463		
Turkish extract v Tap water	18†	29	773	4	Repellent quality of Turkish strain far less than that of Mexican strain
Mexican extract v Turkish extract	26‡	76	1,421	14	
Mexican extract v Untreated cabbage	5	9	510	1	16 of the leaves treated with Mexican extract were not laid on, but all leaves treated with Turkish extract were
Untreated cabbage		65	1,691	25	
Mexican extract v Untreated cabbage	1	12§	13	ND	1978 trial
Untreated cabbage		251§	537	ND	
Mexican extract v Untreated cabbage	1	17§	37	ND	1979 trial
Untreated cabbage		91§	669	ND	
Mexican extract (steamed) v Untreated cabbage	2	38§	143	ND	1978 trial
Untreated cabbage		36§	75	ND	
Mexican extract (steamed) v Untreated cabbage	2	15§	40	ND	1979 trial
Untreated cabbage		122§	546	ND	

*Eggs counted in only 2 and 5 of these trials, respectively. †Eggs counted in 10 trials; egg batches counted in 14 trials. ‡Egg batches counted in 23 trials. §No. of egg batches including singles. Results obtained from female ovipositing in dual choice chamber. Experiments performed by L. Lundgren. ND, not determined.

We sprayed cabbage leaves with aqueous extracts (soups) of plants which do not serve as normal hosts for *P. brassicae*. The ovipositing female will lay sparingly on most of these leaves, totally reject a few, and sometimes lay as freely as on controls sprayed with cabbage extract or tap water. Cabbage (*Brassica oleracea*, greyhound strain) and *Cannabis sativa* (Turkish strain containing predominantly CBD (UNC 354) and Mexican strain containing predominantly THC (UNC 347))¹ were grown under glass at Ashton from seed produced in the UK. Leaves for the experiments were cut from the same plant, matched for size and position on the stem. Butterflies from Brian Gardiner's Cambridge strain² of *P. brassicae* were reared at Ashton.

Aqueous extracts were prepared by macerating 5g of *Cannabis* (or other) foliage in a domestic blender for 5 min, and adding 5oz tap water. The blender was not allowed to warm up. The resulting opaque green fluid was filtered through Whatman No. 1 paper, and a measured amount was sprayed on to each cabbage leaf. Maceration probably liberates unusual volatiles from *Cannabis*, cabbage and any other plant, and such extracts from its own food plant may slightly alter the butterflies' reaction to a leaf (Table 1). In other experiments volatiles were removed by heating the extract slowly after filtration for 30 min until all the characteristic odour had evaporated, leaving only a generalized 'green' or 'vegetable' odour. Lost fluid was replaced by an equivalent amount of tap water, and refiltered to remove green matter that had precipitated during heating; the resulting fluid was a clear brownish yellow. Ethanolic solutions containing 1% CBD and 1% THC were also tested by spraying on to the surface of cabbage leaves.

Two leaves were offered simultaneously to free-flying ovipositing females in a large greenhouse (4.5 × 7.5 m). Placed on reversed flower pots (6–12 inches apart), they were left *in situ* from 1000 h to 1400 h. These pots, with leaves, were transposed at intervals to avoid position effects. In sunny weather the leaves were resprayed twice during the 4-h period. Females were used on the second day of egg laying which resulted in a high proportion of small egg batches.

Aqueous extracts of *Cannabis sativa* sprayed on to the normal host plant drastically reduced egg laying by *P. brassicae*, but the Mexican THC strain proved far more repellent than the Turkish CBD strain. Table 1 shows that, matched against a control sprayed with tap water, egg batches are outnumbered 22:1 on leaves sprayed with Mexican strain, but only 3:1 with Turkish strain.

If well steamed, extracts of both plants, but more especially the Turkish strain, lost their principal oviposition deterrent. In several trials (Table 2) leaves treated with steamed Turkish soup, and once or twice with Mexican soup, attracted more oviposition than the controls, confirming that the steam of volatiles of both plants contained the more active deterrents.

Since slight repellent qualities were usually retained by steamed extract, and some cannabinoids, although in reduced amounts, would occur in the aqueous residue, we tested THC and CBD (dissolved in ethanol) against various controls and each other (Tables 2, 3) and found that 2–3 times as many eggs were laid on leaves sprayed with CBD than THC.

The ovipositing large white is known to react to a variety of visual and sensory cues, including the presence of mustard oils in foliage, egg batches on both leaf surfaces, feeding larvae and injuries to the plants³. It is nevertheless surprising to find that

Table 2 Oviposition on cabbage sprayed with steamed aqueous extracts (soups) from leaves of *Cannabis sativa*

Strains of <i>Cannabis</i> and spray used	No. of trials	No. of egg batches (excluding singles)	No. of eggs laid	No. of times first choice of ovipositing female	Remarks
Steamed Mexican extract v Steamed cabbage extract	11	1 114	ND	All cabbage	1979 trials. Repellent quality of steamed extract was more marked than in 1978
Steamed Turkish extract v Steamed cabbage extract	3	ND	42 96	ND	Less difference between Turkish steamed extract and cabbage
Steamed Mexican extract v Unsteamed Mexican extract	7	ND	638 72	5 0	5 Trials counted. Markedly less deterrent quality in steamed than unsteamed extract
Steamed Turkish extract v Unsteamed Turkish extract	9	ND	405 319	3 1	4 Trials counted. This difference is less marked than between Mexican steamed and unsteamed
Steamed Mexican extract v Steamed Turkish extract	6	ND	11 121	1 5	All leaves with Turkish extract were laid on, but 4 leaves with Mexican extract had no eggs laid on them
Steamed Mexican extract v Steamed Turkish extract	2	0 2	0 11	0 0	Turkish steamed extract preferred to Mexican steamed extract, but many more eggs laid when tap water used
Cabbage and tap water		2	70	4	
Steamed South African extract v Steamed cabbage extract	7	3 (very small) 87	ND	0 7	A THC strain of <i>Cannabis</i> . A huge number of eggs on control leaf on both surfaces

Table 3 Oviposition on cabbage sprayed with purified THC and CBD

Spray	No. of trials	No. of egg batches (excluding singles)	No. of eggs laid	Remarks
THC v CBD	24	21 11 trials counted	1,301 all trials counted	Leaves sprayed with THC always carry fewer batches and fewer eggs than CBD sprayed leaves
THC v Tap water	12	46 145	1,119 6 trials counted	A few trials were made in 1979, and showed a greater contrast than in previous trials
CBD v Tap water	19	100 16 trials counted	3,859 9 trials counted	Fewer egg batches were laid on CBD sprayed leaves. They were sometimes larger than on controls, suggesting an oviposition stimulant but not an attractant
THC v Mexican extract	2	3 0	83 0	Suggests THC less repellent than Mexican extract
THC and Tween v CBD and Tween	8	6 16	176 506	The repellent character of THC compared with CBD is well illustrated in these trials
Tween		8	202	
CBD + ethanol v Ethanol	4	36 37	ND	Ethanol alone is less repellent than either CBD + ethanol or THC + ethanol
Tap water		133		

Table 4 Oviposition on cabbage sprayed with THC and CBD

Spray	No. of egg batches laid			No. of eggs laid			Remarks
	First trial	Second trial	Third trial	First trial	Second trial	Third trial	
THC v CBD	16	9 + 3 singles	8	478	211	ND	In two out of three trials CBD sprayed leaves had more eggs than the control. In one case the THC-sprayed leaf had more eggs laid on it
Cabbage + tap water	16 + several singles	12 + 8 singles	5	678	561	ND	
CBD v Cabbage + tap water	17	12	8	404	383	ND	In both trials CBD-sprayed leaves carried more egg batches and more eggs than the control
	9	4		437	111		
	8	1		350	21		

ND, not determined.

this butterfly can distinguish between the aqueous extracts of two such similar strains of a plant species which does not, in nature, serve as a host. It seems that the major deterrent factors are in the volatiles emanating from these solutions; however, after steaming the extract, there remain non-volatile elements (which have not been identified) in the residue, which influence the ovipositing behaviour of the butterfly. It is therefore interesting to find that the large white can distinguish between two very similar cannabinoids (differing from each other only by a ring closure which changes a substituted terpinoid (CBD) into a dibenzopyran (THC). Although present in small quantities these probably constitute two of the non-volatile repellents in the plants, and the relative unattractive nature of the THC could contribute to the greater repulsive qualities of the Mexican strain.

It is difficult to determine whether the initial choice of a leaf is made after merely a close approach or a rapid or brief contact. It will be seen (Table 2) that out of 26 trials the Mexican extract leaf was only once selected for ovipositing before the Turkish extract leaf. Recognition of a leaf sprayed with CBD and THC occurred after, rather than before 'touch down'.

The tendency for females to lay larger batches (containing more eggs than the controls) on certain CBD-treated leaves

(Table 4) suggests that there may be an ovipositing stimulant, but not an attractant, in this substance. There is no doubt that several different factors contribute to the production of large egg batches, quite apart from the age and past history of the females concerned.

The few results we obtained with Mexican steamed extract in 1979 (Table 2) differed from those of other years. Thus, after heating and steaming, the residue retained much of its original repellent quality. Possibly the THC concentration was higher. Moreover, the spring had been exceptionally cold, and in an unheated greenhouse all the plants suffered. Indeed it would be surprising if the secondary plant substances—always disconcertingly variable^{1,4}—were similar in all respects to those found in the previous crops.

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A curiosity of light adaptation

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By standard psychophysical procedures¹ it is possible to arrange that visual discriminations depend only on signals originating in the violet-sensitive receptors of the eye. A person's vision then shows several characteristic properties: differential sensitivity is lower than when the green- and red-sensitive cones contribute to detection, spatial and temporal resolution is poorer, and a number of anomalies of adaptation reveal themselves^{1–9}, including the saturation that we have demonstrated when violet targets are presented on steady blue or violet adapting fields¹⁰. 'Saturation' refers to the empirical finding that as the intensity (I) of the short-wavelength field increases, the threshold intensity (ΔI) for detecting a violet target rises much more rapidly than is described by Weber's law ($\Delta I/I = \text{constant}$). These earlier results (replicated here; see Fig. 2, right-hand data), were obtained in what would conventionally be regarded as equilibrium conditions, in that thresholds were measured after 4 min of adaptation to the steady field. Here we examine a very odd aspect of the phenomenon: the threshold reaches its saturated state only after passing through a much lower value^{11,12}.

Figure 1 shows (for 3 observers) the time course of the threshold for a 436-nm target during the first 3 min after the onset of a composite adapting field. The latter consisted of a 440-nm component of $10^{10.6}$ quanta $\text{s}^{-1} \text{deg}^{-2}$ and a 575-nm component of $10^{11.7}$ quanta $\text{s}^{-1} \text{deg}^{-2}$. The second, yellow, component would traditionally be termed an 'auxiliary field' (ref. 1, p. 198); it serves to suppress the red- and green-sensitive cones. The left-most data points of Fig. 1 represent the threshold 1 s after the onset of the composite field; the threshold is elevated relative to its dark-adapted value (not shown). Thereafter the threshold falls (as would normally be the case during the first few seconds of light adaptation), passes through a minimum, and then rises with remarkable speed.

For observers J.D.M. and D.D. the function at its steepest has a slope of 1 dBs^{-1} . To record accurately these rapid changes in sensitivity we have revived a special psychophysical method described by Cornsweet and Teller²³. An ordinary staircase procedure¹², such as is traditionally used to track a

changing threshold, would require a very large step size if it were to track faithfully the extremely rapid changes seen in Fig. 1; but then the precision of the estimate at any given delay (Δt) would be low. The modified procedure (called here the 'method of a thousand staircases') is designed to obviate this difficulty. In the present example, the observer receives 13 exposures to the adapting fields, each exposure, or run, lasting 3 min and successive runs being separated by 15-min intervals to allow the visual system to recover. During each run, test flashes (and accompanying warning tones) are presented at 3-s intervals. On the first run of the series the test flashes are all presented at the same intensity and the observer indicates by pushbuttons whether he has seen each flash. The response corresponding to each value of Δt is stored by the computer that controls the experiment. On the next run the computer adjusts the intensity of the flash at a given Δt according to the response previously given at that Δt but independently of responses given earlier in the current run. The new response at each Δt is now stored and is used to determine the flash intensity at that Δt on the third run; and so on. The adjustments between runs are made according to the rules for a single staircase¹³, but what is unusual is that a large number (here 60, but in principle unlimited) of staircases are being maintained concurrently, each staircase corresponding to a single Δt . The final thresholds plotted in Fig. 1 are based on the intensity levels visited at a given Δt in the last eight runs.

Figure 2 places the phenomenon of Fig. 1 in context. Consider first the data to the right. These constitute an increment-threshold function ('threshold-vs-intensity' (t.v.i.) curve) for 436-nm targets presented on a 440-nm field and were obtained in steady-state conditions, in that measurements at each intensity of the field were preceded by 4 min of adaptation to the field. A fixed yellow (575 nm) auxiliary field of $10^{11.2}$ quanta $\text{s}^{-1} \text{deg}^{-2}$ was present throughout. The broken line has a slope of unity and corresponds to Weber's law. The thresholds deviate upwards from this line, showing the operational saturation described earlier. At the highest intensity of the 440-nm field, another class of cones becomes responsible for detection¹⁰ and the function flattens. A conventional, random double-staircase procedure¹³ was used to obtain these data.

In the central panel of Fig. 2, log threshold is plotted as a function of Δt , with the intensity of the 440-nm field as parameter. These data were obtained by the method of a thousand staircases and they show how the threshold reaches its final 'equilibrium' value. The broken lines project the

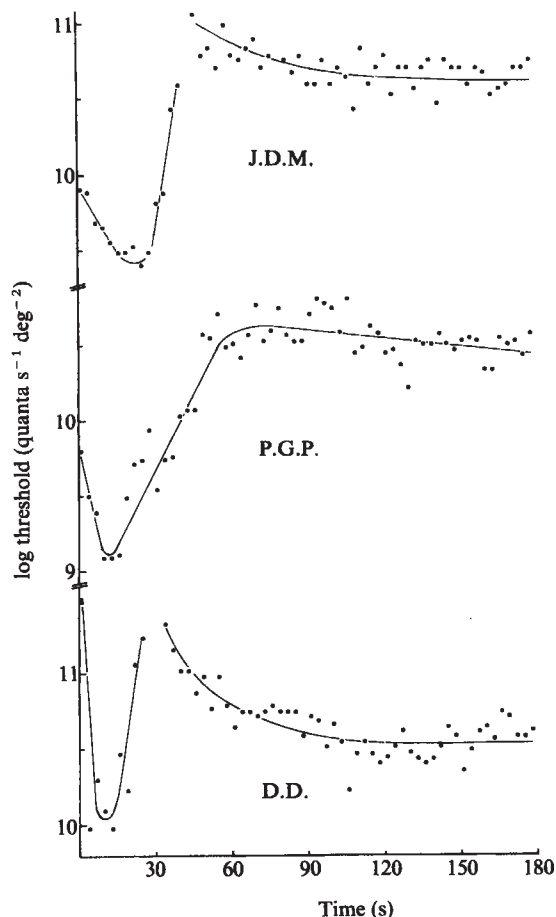


Fig. 1 Variation of log threshold intensity for detecting 436-nm flashes during the first 3 min of adaptation to a composite adapting field consisting of a 440-nm component at an intensity of $10^{10.6}$ quanta $s^{-1} \text{deg}^{-2}$ and a 575-nm component at an intensity of $10^{11.7}$ quanta $s^{-1} \text{deg}^{-2}$. (The nominal chromaticity coordinates of this composite field are $x=0.45$, $y=0.47$.) The target flash subtended 1 deg of visual angle, was delivered to the centre of fovea and had a duration of 200 ms; the steady adapting fields were concentric with the target flash and subtended 6.5 deg. Target flashes were presented at 3-s intervals, each being preceded by a warning tone. A period of 2 min of dark adaptation preceded the onset of the fields. The solid lines are interrupted at values of Δt at which insufficient light was available to measure a threshold. Observers are denoted by initials.

asymptotic threshold levels to their rightful places on the t.v.i. curve. The 575-nm field remained the same intensity throughout and was combined in different sessions with different intensities of the 440-nm field.

The behaviour of the threshold changes systematically with small changes in the intensity of the 440-nm field. In all cases, the threshold passes through a minimum before rising again, but the more intense the field the earlier the minimum occurs and the steeper the slope of the rising part of the function. The latter trend can be seen in the data of Stromeyer *et al.*¹². Ancillary evidence suggests that for $\Delta t > 45$ s the upper limit to the family of functions in Fig. 2 depends on detection by a recovering long-wavelength mechanism (see also ref. 12, Fig. 14). At the lower field intensities, the functions resemble those given by Augenstein and Pugh⁸ for light adaptation to long-wavelength fields alone. The left-most panel of Fig. 2 is discussed below.

Hitherto, we have treated the 575-nm field merely as an auxiliary field, serving to adapt the long- and middle-wavelength cones. If we assume that the spectral sensitivity of the short-wave receptors is approximately that of the

psychological mechanism Π_3 (ref. 1, p. 18), then in our conditions the 575-nm field will contribute never more than 1% to the total number of photons absorbed in those receptors. Yet Fig. 3 demonstrates that this field (and thus probably a signal originating in the long- and middle-wavelength cones) is crucial to the temporal variation in sensitivity. In the control condition of this experiment (solid circles, Fig. 3) the onsets of the fields were simultaneous as before, but in the experimental condition (open circles) the onsets were uncoupled, the onset of the 575-nm field being delayed by 40 s relative to that of the 440-nm field. The delay chosen is equal to a Δt at which the threshold would have passed its minimum if the onsets of the fields had been simultaneous. Yet 40 s of pre-adaptation to the violet field has little effect on the function: the onset of the delayed yellow field finds the threshold close to the value for $\Delta t = 1$ s in the control condition and the threshold then passes through its curious oscillation as before; the rising part of the function is displaced along the abscissa by a delay (47 s) that is slightly greater than the imposed delay of the yellow field. Because it seems to matter only slightly whether or not the retina has been pre-exposed to the 440-nm component, the phenomenon must depend substantially on a signal originating outside the short-wavelength receptors. However, the 440-nm component (and thus, probably, the adaptive state of the violet-sensitive receptors) must be independently important, as variation in its intensity produces the family of functions seen in Fig. 2. The 7-s discrepancy noted in the present results may be attributable to the difference in the adaptive state of the short-wavelength receptors.

We relate the present phenomenon to a general model of the short-wave mechanism^{14,15}, in which it is supposed that signals originating in the short-wavelength receptors are transmitted only via chromatically opponent pathways¹⁶⁻¹⁸ and that the sensitivity of the psychophysically defined short-wavelength mechanism depends on (1) the adaptive state of the short-wavelength receptors and (2) the balance of short- and long-wavelength signals reaching a chromatically opponent site. A crucial assumption is that a chromatically opponent channel is most sensitive to input perturbations when it is close to the centre of its operating range; as it is 'polarized' (that is, driven to one or other end of its operating range by, for example, a saturated blue or a saturated yellow field), incremental or decremental inputs are increasingly attenuated. In our experiments, yellow fields alter the measured sensitivity to short-wavelength targets primarily by altering the polarization of the opponent site, for they negligibly stimulate the short-wavelength receptors. The violet fields act both at short-wavelength receptors and at the opponent site, but, in the presence of the yellow field, contribute negligibly (<5%) to absorptions in the long- and middle-wavelength cones. Note that the most sensitive state of the opponent channel almost certainly does not correspond to the condition of equal rates of isomerization in the three classes of receptor; there is good evidence that the signal from the violet-sensitive receptors is disproportionately weighted in its contribution to this channel¹⁹.

As a working hypothesis we suppose that the present phenomenon arises from a temporal variation in the signal delivered to the opponent site from the long- and middle-wavelength cones. A 575-nm field of $10^{11.2}$ quanta $s^{-1} \text{deg}^{-2}$ has a troland value of $10^{5.0}$ and will bleach approximately 85% of the pigment in these cones²⁰; most of the change in the proportion of unbleached pigment will occur in the first minute after onset of the field²¹. During this time the signal reaching the opponent site from these cones will presumably fall rapidly, owing both to bleaching and to other processes of receptor adaptation (see, for example, ref. 22). The photo-sensitivity of the short-wavelength cones is not known but our calculations suggest that they will not be substantially bleached by our 440-nm field. So we assume that during light adaptation to our composite field the signal from the long- and middle-wavelength cones is changing more rapidly than

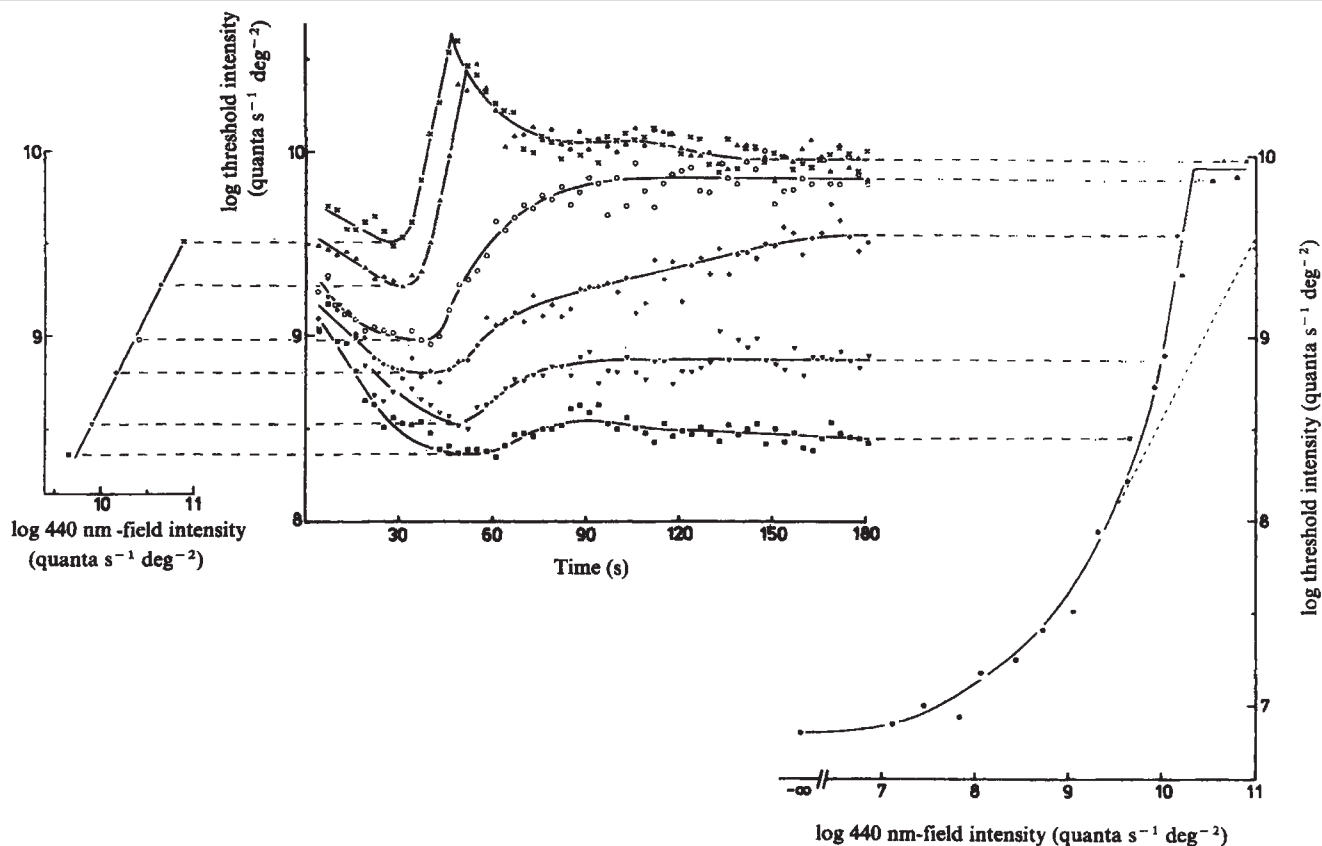


Fig. 2 Right-hand side: a conventionally obtained increment-threshold function for 436-nm test flashes presented on steady 440-nm fields of increasing intensity. A 575-nm auxiliary field of fixed intensity ($10^{11.2}$ quanta s^{-1} deg^{-2}) was present throughout. Thresholds were measured using a randomized double-staircase procedure and the plotted values are based on a sequence of 32 responses obtained between 3.5 and 5.5 min after onset of the composite adapting field, earlier responses being used to place the staircases close to the estimated threshold. Stimulus parameters were as for Fig. 1. Observer: J.D.M. Centre: temporal course of the threshold for detecting 436-nm test flashes during the first 3 min of adaptation to a series of composite fields consisting of a fixed 575-nm component of $10^{11.2}$ quanta s^{-1} deg^{-2} and 440-nm components of intensities: $\times 10^{10.88}$, $\blacktriangle 10^{10.63}$, $\circ 10^{10.39}$, $+ 10^{10.14}$, $\nabla 10^{9.88}$, $\blacksquare 10^{9.64}$ quanta s^{-1} deg^{-2} . Two experimental sessions were devoted to each of the latter values and the order of sessions was randomized. Left-hand side: minimal thresholds as a function of the log intensity of the 440-nm field. The solid line has a slope of unity.

that from the short-wavelength cones. The opponent site passes from being polarized in the yellow direction to being polarized in the other direction and the threshold, correspondingly, falls, passes through a minimum, and then rises again.

A further feature of the parametric results of Fig. 2 (central panel) is compatible with the hypothesis outlined above. The minima of the several functions should correspond to instants at which the opponent site is in the same (maximally sensitive) state. Differences between the minimal thresholds must then depend only on variations in sensitivity at more distal sites in the channel that carries the signal. In the left-most panel of Fig. 2 we plot the minima against the intensity of the 440-nm field: these values, unlike the asymptotes (right-most panel), obey Weber's law closely. As Weber's law (whatever its ultimate explanation) characterizes the threshold for large achromatic targets presented on bright steady fields, it is the behaviour that might be predicted when we hold constant the state of the opponent site. In other experiments we have held constant the 440-nm component of the field and varied the 575-nm component: in this case the minimum threshold, though reached at different values of Δt , varies little in its absolute value, as would indeed be predicted if (1) sensitivity at the distal site is predominantly set by the 440-nm component and (2) the minima represent equivalent states of the opponent site.

Is the hypothesis supported by the observer's

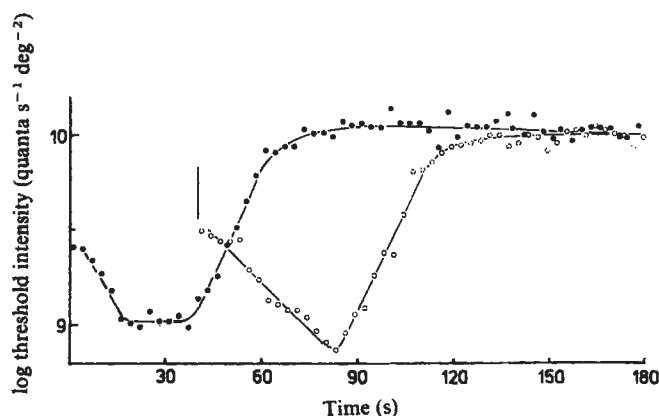


Fig. 3 Solid points: variation of log threshold intensity for detecting 436-nm test flashes during the first 3 min of adaptation to a composite adapting field consisting of a 440-nm component at an intensity of $10^{10.4}$ quanta s^{-1} deg^{-2} and a 575-nm component at an intensity of $10^{11.2}$ quanta s^{-1} deg^{-2} . Observer: J.D.M. Open points: results obtained when the onset of the 575-nm component of the field was delayed by 40 s. All points are plotted relative to the time of onset of the 440-nm component of the field. The onset of the delayed yellow field is indicated by a vertical bar.

phenomenological experience? Assuming that it is the central region of the field that is most relevant, the CIE chromaticity coordinates of the composite fields used for the measurements of Fig. 2 range from $x=0.47$, $y=0.50$ to $x=0.36$, $y=0.32$; but these chromaticities are not a good guide to the appearance of intense stimuli in transitional conditions. Formal phenomenological reports were obtained in separate runs in the conditions of Fig. 2. At onset, all fields were 'dazzlingly' bright and they varied in hue from 'yellow' to 'pale violet' according to the intensity of the 440-nm field. In all cases, after about 10 s, the field darkened strikingly and turned a grey or slate blue colour. After 60 s the colour was always pinkish, and, after 120 s, greyish. The sudden darkening always occurred a few seconds before the independently measured loss of sensitivity, and the value of Δt at which it occurred varied less with field intensity than did the point of minimum threshold. We therefore hesitate to relate the phenomenology to the processes, probably retinal, revealed by the sensitivity measurements; and the reader will have noticed that throughout this article we have avoided the term 'blue-yellow opponent channel'.

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Visual acuity is better for letters in rows than in columns

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The spatial resolving capacity of the visual system is usually measured with charts containing letters of different sizes displayed in horizontal rows or, less often, in vertical columns. Although previous studies suggest that acuity may not be equal for these two arrangements¹⁻⁷ it is implicitly assumed in clinical tests that orientation of an array of letters is of no consequence to the measurement. The work reported here shows that this assumption is incorrect. While undergoing an examination in an eye clinic, I noticed that letters of a given size seemed more difficult to identify when they were arranged in vertical compared with horizontal arrays. Subsequently, I tested a group of subjects using letters presented pseudo-randomly with different inter-letter spacings, in rows or columns. Subjects were required to identify letters from left to right or from top to bottom in each isolated row or column. A significantly higher number of errors was made for letters in columns than for those in rows. The effect does not depend on order or direction of letter presentation, and it is not found when acuity determinations are made using a non-letter test symbol. To explore the conjecture that the effect could be associated with previous experience, two additional groups of subjects were tested. First, bilingual Chinese, whose first language is read in both horizontal and vertical arrays, were tested using Chinese characters and English letters. Second, acuity determinations were made with young children who could identify letters but did not read. In each case, acuity was not significantly different for horizontal or vertical presentations, a finding consistent with the notion that learning may influence the development of visual resolution.

The tests were carried out with slides containing pseudo-random combinations of eight letters—E, H, R, N, O, X, D and V—arranged in groups of columns or rows. Each letter appeared once at a given inter-letter separation (see below). The slides were mounted in frames for use in a standard vision chart projector. Before the tests, the refractive condition of each subject was carefully determined. Subjects were rejected if

a visual anomaly or considerable refractive error was found. Using appropriate ophthalmic lenses if required, subjects monocularly viewed letters that each initially subtended an anticipated threshold level of 4 arcmin at the eye. (A 7.1-mm letter viewed at 20 ft subtends 4 arcmin and if correctly read, an acuity of 20/15 is indicated.) Letters were presented in a column or row and all eight letters were shown at each of five inter-letter separations (2, 4, 8, 16, and 32 arcmin). For each subject, the zoom lens on the projector was adjusted so that letters were small enough to be near threshold, as estimated by approximately 75% correct identification. Letters of all separations were then systematically presented in columns or in rows and subjects were asked to identify them from top to bottom or from left to right, respectively. Half of the subjects were tested using columns followed by rows and the other half were tested in the reverse order. No differences were found related to order of presentation. Ten letters were used at each inter-letter spacing, but only the inside eight were scored to avoid possible complications of edge effects at the ends of each row or column.

Results for 11 subjects, given in Fig. 1a, show that for both columns and rows, the error rate increases with decreased separation between letters, as could be anticipated from previous work⁸. However, in addition, the mean error rate is clearly higher for columns than for rows of letters and the overall difference is highly significant ($\chi^2=26.12$, $P=0.0062$).

What can account for this difference in acuity between horizontal and vertical arrays of letters? An optical explanation of the effect can be ruled out immediately. The subjects used did not have high amounts of ocular astigmatism, and the moderate or small astigmatic errors found were of both vertical and horizontal types. In either case, these errors were fully corrected with lenses. Furthermore, when grating test targets are used to determine acuity, no differences are found between vertical and horizontal orientations^{9,10}. This applies also to laser-created interference-fringe gratings for which the effect of the eye's optics are essentially bypassed¹⁰.

One must therefore propose an alternative explanation for the effect found here and an obvious possibility is that the development of visual acuity for arrays of letters is associated with learned patterns of reading. Specifically, because we learn to read rows of letters, finest acuity develops correspondingly for horizontal displays. Because we also learn to read from left to right, acuity may be superior for this direction compared

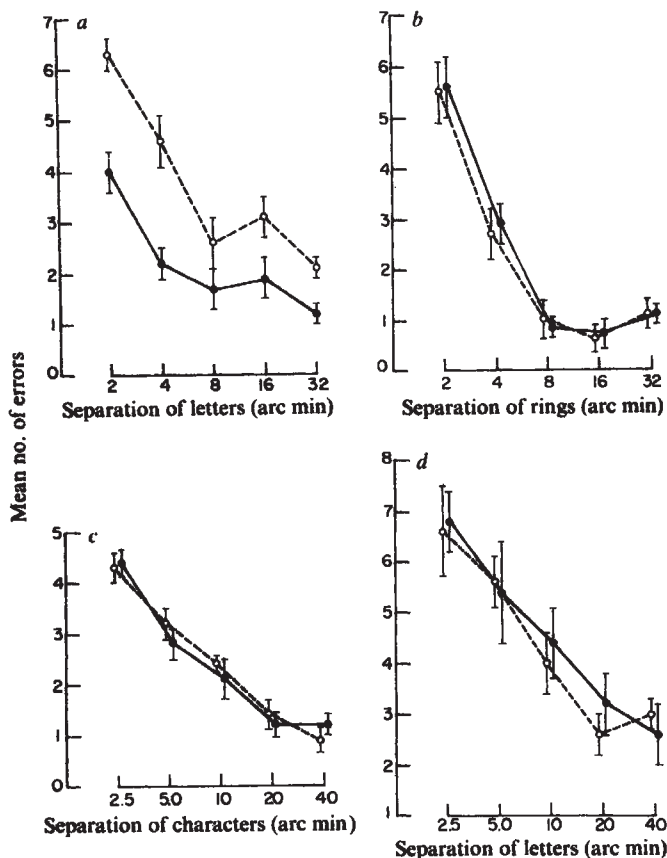


Fig. 1 Results are given for visual acuity tests using letters with adults (a) or children (d), Chinese characters with Chinese subjects (c), or Landolt rings (b), presented in vertical columns (open symbols, dashed lines) or horizontal rows (filled symbols, solid lines). Each point represents the mean number of errors for eight presentations at given inter-letter (or character or ring) separations. Vertical bars represent ± 1 s.e.m. Abscissae are scaled logarithmically.

with the reverse. To examine this possibility, a second group of subjects, most of whom were included in the first group, was tested with the same letter chart used before. Only, in this case, letters were identified from right to left in rows or from bottom to top in columns. Results were very similar to those shown in Fig. 1a, with a significantly higher number of errors found for columns than for row arrays. The acuity effect is therefore selective for orientation but not for direction. If there is a direction-selective component, a more sensitive measure which might incorporate temporal factors is required for its study.

The hypothesis linking visual acuity development and experience applies to letters that must be recognized. For simpler acuity targets that require detection only, learned reading habits do not apply and therefore, differences between rows and columns would not be expected. To determine this, acuity was measured using Landolt rings, which contain a gap at the top, bottom, left or right whose subtense is 1 arc min at 20 ft. These were presented in rows or columns with inter-ring separations, just as in the previous case. The same group of subjects, whose letter acuities are shown in Fig. 1a, were tested using Landolt ring targets and the results are given in Fig. 1b. Clearly, there is no difference between acuities for rings in rows as compared with columns. In both cases, the data show a very high mean error rate for closely spaced rings, suggesting that this type of detection task may be more difficult than letter identification when test arrays are dense. It thus seems

that acuity differences between rows and columns of test objects may apply specifically to letters; this is consistent with the idea that they are related to learned reading patterns.

It would be possible to test this notion more directly by measuring acuity of subjects whose language is read and written primarily in vertical rather than horizontal arrays. In this case, acuity would be expected to be reduced for rows instead of columns of letters. However, there are apparently no common languages that are arranged only in vertical columns. On the other hand, modern Chinese is read and written in both columns and rows so that no bias would be expected on the basis of learned reading patterns. Ideally, it would be best to test Chinese subjects who do not read or write English. This was not practical, but it was possible to measure acuities of bilingual Chinese subjects for whom English is a second language learned during teenage years. Following thorough refractions, acuities were determined using eight standard Chinese characters, chosen for simplicity, which each subtended 5 arc min initially. Inter-character separations were 2.5, 5, 10, 20 and 40 arc min. In addition, acuities were also determined with this group using the English letter chart. The results for characters, presented in Fig. 1c, clearly show that there are no acuity differences between rows and columns (confirmation is provided by the χ^2 statistic). Results with this same group using English letters showed a tendency for an increased number of errors for column arrays, but this trend is not significant (χ^2 statistic).

Although unlikely, it could be argued that there are inherent racial differences in visual resolving capacities between these Chinese subjects and the American group tested previously. The possibility is easy to rule out, however, by measuring acuity for English letters using Chinese-American subjects who have never learned to read or write characters. Five subjects in this category were tested with the same chart as used for the initial group of Americans. The results were virtually identical to those for the latter group and it may thus be concluded that inherent racial factors are not involved in the findings described here.

One additional group of subjects was tested. If an acuity bias for horizontally arranged letters develops as a result of learned reading patterns, subjects who know the alphabet but do not yet read should not demonstrate a difference between rows and columns of letters. Five children, aged 4–5 y, were refracted and then shown the eight letters used on the chart to insure that they could identify them. Acuities were then determined, the results for which are shown in Fig. 1d. As might be expected, variability is greater than that for the other groups, but there are no consistent differences between rows and columns.

Taken together, the results of this study suggest that visual acuity performance for letters is related to reading patterns learned in childhood. The finding which is not compatible with this idea is that the acuity difference between rows and columns holds for right to left identification as well as the reverse. It is possible, of course, that a directional effect could be found using different tests. In any case, the neural mechanism that underlies the relatively high acuity for horizontal arrays may be concerned primarily with fixation. For example, contours adjacent to a letter that interfere with its resolution^{11,12} may be more effectively masked in row arrays. The advantage of horizontal letter displays, in terms of acuity, may be relevant to other work showing that reading speed is slower^{3,4} and word or pseudo-word identification poorer^{5–7} for vertical compared with horizontal arrangements of letters. Further study is required to determine the relative importance of factors such as spacing and numbers of letters in a given array. In addition, it would be interesting to know if eye movement patterns differ for reading in row as opposed to column arrangements.

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Eye movement in strabismic cats

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Strabismus is a common clinical condition in which the visual axes of the eyes do not intersect on the object being viewed¹. As such, the ability to achieve single binocular vision by fusing the images of a single object in the two eyes is lost. In most cases of strabismus, one of the two eyes is clearly deviated and the other eye is used for fixation, although in some cases each eye is used alternately for fixation^{2,3}. While much attention has been devoted to the motor capabilities of the deviating eye in strabismus^{1–3}, little attention has been given to the visuomotor competence of the other eye. We report here that, if one eye of a kitten is made to deviate by surgery, the visuomotor capacities of the other, 'normal', eye are affected. A reduction in the ability to follow the movement of a large striped drum is observed with binocular viewing, even when stimuli are viewed monocularly with the normal eye. This means that anomalous visual input from the deviated eye during stimulation is not the cause of the reduced oculomotor capacities.

Divergent strabismus was induced in three 10-day-old kittens by severing the medial rectus muscle of the right eye. The left eye was not touched. The kittens were then reared normally in a well-lit laboratory environment until they were 8–12 months old. At this time the normal undeviated eye of the cat was prepared for eye movement recording by implanting a magnetic search coil according to the technique of Robinson⁴. In addition, head restraining tubes were attached to the skull by means of dental acrylic⁵. Surgery was performed under Alfathesin anaesthesia and the animals were allowed at least 2 weeks to recover before recording began. The eye movements of the three strabismic cats were compared with those of three normal cats studied with the same techniques.

We primarily studied eye movements evoked in response to visual and vestibular stimuli in the horizontal plane. When a large striped drum is rotated about a cat, a characteristic pattern of eye movement called optokinetic nystagmus (OKN)⁶ is evoked with a time course resembling a sawtooth. The slow phase of this response matches the velocity of the drum in normal cats and thus compensates for the motion of the drum and permits clear vision. Figure 1 compares the velocity of the slow corrective phase of optokinetic nystagmus (measured in the nondeviating eye) of strabismic cats with that

of normal cats as a large striped drum is rotated about the animal at various velocities. The results show that the normal cat is able to match the velocity of his eyes to that of the drum rather well over most of the velocity range tested.

By contrast the velocity match of the apparently normal eye of the strabismic cat with that of the drum is much less accurate. For all drum velocities above 4° per s, the eye rotated more slowly than did the drum. This lessened response to visual stimulation is unlikely to be due to any generalized deficit in oculomotor capacity, due, for example, to mechanical impediments which might have resulted from implantation of the eye coil or from the early surgical procedures on the other eye. The eye movement responses to vestibular stimulation as assessed by rotating the cat sinusoidally in the dark were the same in normal and strabismic cats. In both normal and strabismic cats, the vestibulo-ocular reflex was able to compensate for the head and body movement induced by rotating the cat^{5,7}. Thus, the deficit in oculomotor capabilities seems to be specifically related to the response to visual stimuli.

The results illustrated in Fig. 1 were obtained with both eyes open and did not permit assessment of the contribution of vision with each eye to the reduced oculomotor response of the nondeviating eye. Accordingly we measured optokinetic responses of the 'normal eye' to visual stimuli presented through either eye. The interpretation of the monocular stimulation findings (Fig. 2) is rendered more difficult by the finding that monocularly-viewed stimuli moving medially (that is, rightward viewed through the left eye) were more effective at eliciting OKN than lateral-directed stimuli even in normal cats⁸. This difference in the effectiveness of medial and lateral-directed stimuli was also observed with monocular stimulation of the nondeviating eye in the strabismic cats. It is clear, however, that the efficiency of OKN was decreased markedly for both medial and lateral stimulus motion, relative to that of the normal animals. This defect in monocularly-evoked OKN rules out anomalous visual input through the deviating eye during the stimulation period as a possible cause of the reduced OKN observed with binocular stimulation. Visuomotor performance mediated by stimuli presented through the deviating eye was also severely impaired. Figure 2b shows optokinetic responses of the nondeviating eye to visual stimuli viewed monocularly through the deviating eye. These data show that reduced OKN followed stimulation of the deviating eye when stripes moved in either direction. The

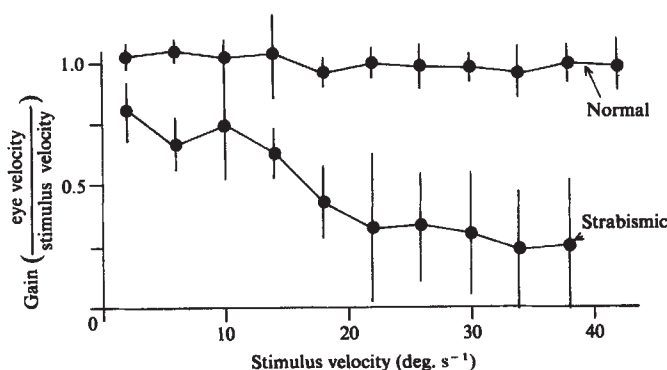


Fig. 1 The gain of optokinetic nystagmus as a function of drum speed for the normal and strabismic cats. The gain of the response is the velocity of the slow phase⁶ of the optokinetic response divided by the velocity of the drum. A gain of 1 indicates perfect following of the drum by the eyes. The gain of the normal cats is close to 1 over most of the velocity range tested while that of the strabismic cats is markedly lower. The drum consisted of a large cardboard cylinder 90 cm in diameter on which high contrast black and white vertical stripes (period 20°) were painted. Background luminance was 0.1 cd m⁻².

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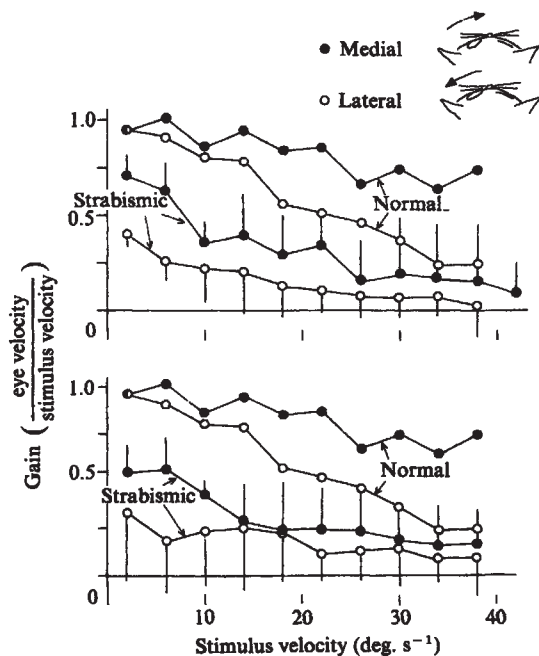


Fig. 2 *a*, The gain of the slow phase of optokinetic response in normal and strabismic cats when stimuli are viewed with the nondeviated eye only. The gain of the reflex is lower for laterally-directed high velocity drum movement than medially-directed movement in both the normal and strabismic cats. The gain in both directions is considerably lower for the strabismic cats than it is for normal cats even though the strabismic animals viewed the stimulus through the nondeviated eye. In normal cats, the gain of the reflex is the same regardless of whether the monitored eye or the other eye alone views the stimulus, as would be expected from Hering's Law¹⁶. *b*, Responses to drum motion when the strabismic animals view through the deviating eye alone are compared with those of normal cats viewing monocularly. The deficits observed in the visuomotor capacities of the strabismic cats are similar regardless of which eye views the moving stimulus. The standard deviations (s.d.) have been omitted from the curves representing responses of the normal cats for clarity. A typical value for the s.d.s in this case is 0.15. Drum parameters are as in Fig. 1.

deficits of OKN were similar with stimulation of either the deviating eye or the nondeviating eye.

The present results show that the visually-evoked eye movements mediated through an apparently normal eye can be altered by surgical exodeviation of the other eye. The eye movement deficit seems to be specific to visual stimulation as vestibular stimulation evoked normal oculomotor responses. The mechanism underlying this reduced visuomotor competence remain unknown but several anomalies identified in various parts of the visual system in strabismic cats⁹⁻¹¹ help towards its understanding. We have recently recorded visual responses in the nucleus of the optic tract (NOT) of strabismic and normal cats¹¹. Our findings in this structure, which has an essential role in optokinetic nystagmus^{12,13}, indicate marked changes in binocular connectivity among NOT neurones following surgically-induced strabismus. These neural changes may provide a basis for the visuomotor deficits found in the strabismic cats. It would be interesting to see if similar defects exist in the normal eye of human strabismics, particularly in view of the recent findings suggesting defects on OKN in the normal eye of amblyopic humans^{14,15}.

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Genetic control of sensory connections in *Drosophila*

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The function of the nervous system depends on the formation of a net of appropriate connections. This process must be at least partly under genetic control, yet the genetic analysis of the development of specific nerve connections has so far made little progress (reviewed in ref. 1). This is in part because of the immense complexity of the connective net. The problem is simpler in the case of insect secondary neurones which are derived from the epidermis and send their axons centripetally towards the central nervous system². The arrangement of sense organs on the body surface is very reproducible in many cases, so that given neurones can be recognized unambiguously in different individuals. In *Drosophila*, the genetic analysis of development has so far concentrated on the epidermis. The genetic control of segmentation is relatively well understood^{3,4} and it has been found that segments are progressively subdivided in smaller developmental units called compartments⁵. This led to the speculation that sensory neurones belonging to different compartments might have different properties⁶. Here we show that the stimulation of mechanoreceptor bristles at different positions on the notum and legs of *Drosophila* specifically evoke different behavioural responses. This specificity depends on the segmental and in some cases on the compartmental identity of the bristle, but not on the site of entry of the axon into the central nervous system.

Decapitated flies remain alive and standing for up to 20 h. They remain motionless if left undisturbed, although they are able to stand up if overturned, or to walk a few steps if pushed. The tickling of single bristles induces several reactions: wing scissoring, withdrawal of the stimulated body part, and vigorous cleaning by a leg. The latter reaction was analysed in more detail, for it provides a specific and easily scored response. The response evoked by the different macrochaetes on the humerus and notum is quantified in Fig. 1. The microchaetes on the notum were also examined, but their small size and high density makes it impossible to stimulate them individually. It appears from Fig. 1 that the notum is divided in two regions, one where all the bristles evoke a response of the first (and to some extent of the second) leg, and the other which stimulates the third leg. It should be noted that no bristle evoked specifically a response of the second leg, but that such a response was observed in many cases as an alternative to the response of the first leg, mostly after the latter had already responded repeatedly to

continuing stimulation of a given bristle. In order to characterize the specificity of the response further, we have analysed two mutations which result in the development of a supernumerary notum at an ectopic location. The mutation *wingless* transforms the wing to a notum in mirror image symmetry to the normal notum⁷. In this case the axons from the ectopic bristles join those from the normal bristles and enter the thoracic ganglion at the normal place⁸. We have confirmed that the ectopic bristles induce the same response as their normal homologues⁸. The double mutation *bithorax*³ *postbithorax* transforms the bare, vestigial metanotum into a mesonotum bearing the whole complement of notal bristles⁹.

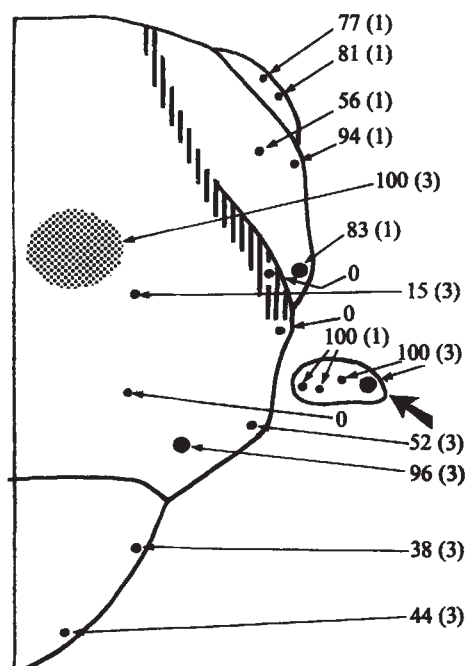


Fig. 1 Frequency and specificity of response to stimulation of the bristles on the notum and tegula. Flies of wild type Canton S strain were exposed to ether, decapitated and left to recover for 1–2 h in a moist environment. Stimulation was achieved by gently touching individual bristles with a hair. A micromanipulator was used in some cases. The numbers give the proportion of flies (out of 48) where a given bristle evoked a cleaning movement by a leg. The numbers in parentheses indicate which leg responded (1: prothoracic leg, 3: metathoracic leg). Bristles represented by large dots were selected for the analysis of the homoeotic situation in Fig. 2. The tegula is drawn at the side of the notum (arrow). The dotted area represents the region where groups of microchaetes were stimulated. The dashed stripe separates the zones which evoke responses of the first and third legs respectively.

Here the axons from the ectopic bristles enter the thoracic ganglion much posterior to the normal place¹⁰. The stimulation of these ectopic bristles gives a pattern of responses which is identical to the pattern of the normal mesonotum both in the specificity and in the frequency of the responses (Fig. 2).

The bristles on the legs were also characterized (Table 1). As for the microchaetes on the notum, most of the leg bristles are too small and dense to be stimulated individually. However it appeared that groups of microchaetes on the anterior and posterior regions of the first and third legs give different responses, and therefore the bristles of the two regions have been listed separately in Table 1. We have attempted to define more precisely the limit between the leg bristles which give an 'anterior' response and those which evoke a 'posterior' response in the case of the femur and tibia of the first and third legs. The results are shown in Fig. 3; the figure also shows the boundaries separating the anterior and posterior

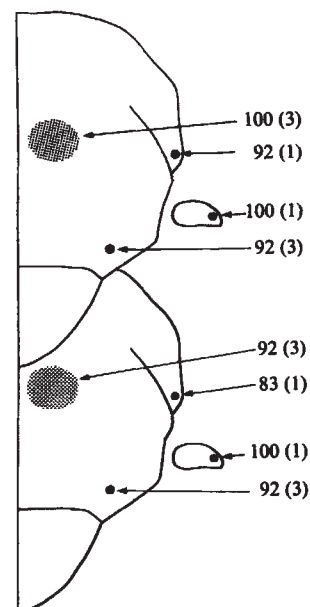


Fig. 2 Frequency and specificity of the responses in the mutant *bx³pbx/Ubx¹⁰⁵*. The normal mesonotum is above, the transformed metanotum is below. The bristles marked by large dots on Fig. 1 have been selected because they give a high frequency of response. The numbers are as in Fig. 1, except that 12 flies were analysed.

developmental compartments of these legs¹¹. The coincidence between the sensory and the compartmental boundaries is obvious.

There is a second situation where an apparently homogeneous population of bristles is divided by a compartmental boundary. The limit between the thorax and wing compartments runs through the tegula, a sclerite at the base of the wing. It is remarkable that within this small area, two of the bristles evoke an intense response of the first leg while two others induce a movement of the third leg (Fig. 1, arrow). However, in this case the identity between sensory and compartment boundaries cannot be assessed, since the exact position of the compartment boundary has been reported as variable within the tegula¹².

Table 1 Frequency and specificity of responses to stimulation of leg bristles in wild-type *Drosophila*

	wtd	2nd	contra
AFe 1	83		33
PFe 1	13	92	
ATi 1	63		46
PTi 1	71	21	4
AFe 2	100		
PFe 2	100		
ATi 2	100		
PTi 2	100		
AFe 3	33	88	4
PFe 3	71		15
ATi 3	79	21	
PTi 3	63		25

The left column indicates the stimulated area. AFe and PFe: anterior and posterior femur; ATi and PTi: anterior and posterior tibia; 1, 2 and 3: first, second and third legs. The next three columns represent the three responses which have been scored. wtd: withdrawal of the stimulated leg; 2nd: cleaning of the stimulated leg by the second (mesothoracic) leg; contra: cleaning of the stimulated leg by the contralateral leg. The numbers represent the per cent of flies (out of 48) where a given stimulation evoked a given response. The numbers add up to more than 100 because the stimulation of a given set of bristles may evoke different responses.

Our results on wild-type flies indicate that the connections established by a sensory neurone depend on its position in two different ways. In the case of the legs, the specificity of the response clearly depends on the segmental identity of the neurones. Furthermore, within each leg, groups of bristles with different specificities can be detected. In those cases where a detailed analysis could be done, the limit between groups of bristles which evoke different responses appeared to coincide with the position of the anterior-posterior compartment boundary. The same is probably true in the case of the tegula, although the position of the compartment boundary is not as precisely defined as it is in the legs. These results show that the choice of a given pattern of connections depends on the segmental and compartmental identity of the neurone. Recent experiments have shown that the establishment of a given projection in the central nervous system also depends on the developmental history of the sensory neurone in the locust¹³, and on the segmental and compartmental identity of these neurones in *Drosophila*¹⁴.

In the case of the notum, the different specificities of the anterolateral and dorsoposterior bristles must depend on something other than the compartmental identity since all these bristles belong to the same compartment. In this case, the decision to establish a given type of connection appears to depend on the position of the bristle along the anteroposterior (and possibly mediolateral) axis of the body. A similar dependence of the evoked behavioural response on the position of the sense organ along a body axis has been reported in the pupa of *Sphinx ligustri*¹⁵. Our results indicate that the neurones retain their specific pattern of connections even when they enter the central nervous system at an abnormal place. This may be related to the fact that ectopic or misrouted neurones which enter the central nervous system at an abnormal place can nevertheless establish a normal projection¹⁰.

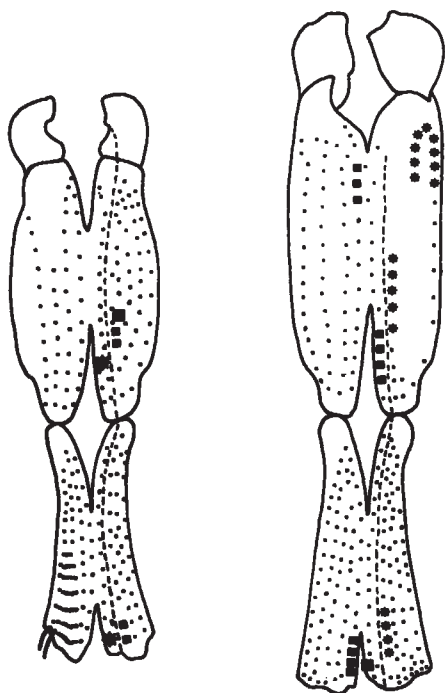


Fig. 3 Response of single bristles or rows of bristles on the prothoracic (left) and metathoracic (right) legs. All the leg bristles are represented by dots. Bristles which induce a withdrawal of the leg or a cleaning response of the contralateral leg are marked with asterisks, bristles which induce a cleaning movement of the mesothoracic leg are marked with solid squares. The broken line shows the position of the anterior-posterior compartment boundary. The chaetotaxy of the legs and position of the boundary are from ref. 11.

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Testosterone-sensitive neurones respond to oestradiol but not to dihydrotestosterone

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Testosterone is aromatized to oestradiol in the brain, and it has been suggested that its effects on the brain are produced by this metabolite¹. Oestradiol does indeed restore sexual behaviour in castrated rats^{2,3}, but it is much more effective if combined with a second metabolite, dihydrotestosterone^{4,5}. This may be because the two hormones have a synergistic effect on the brain; or because the central effect of oestradiol synergises with the peripheral effects of dihydrotestosterone (which include stimulation of penile development). Evidence that the corticomedial amygdala (CMA) and the preoptic area of the rat brain are both involved in the control of sexual behaviour led us to examine the neurones of the stria terminalis that connect the two^{6,7}. These neurones are hormone-sensitive; their absolute refractory period is lengthened by castration and reduced again by testosterone. So we have investigated the aromatization hypothesis further by examining the effect of oestradiol and dihydrotestosterone on these testosterone-sensitive neurones. Oestradiol has exactly the same effect as testosterone; dihydrotestosterone has no effect, whether it is given alone or in combination with oestradiol.

Thirty-two virgin, adult male Wistar rats (400-650 g) were divided at random into four groups of eight and castrated under ether anaesthesia. Eight weeks or more after castration, rats in each group were assigned to treatment with (1) 5 µg oestradiol benzoate (OB), or (2) 1 mg 5α-dihydrotestosterone propionate (DHTP), or (3) 1 mg DHTP + 5 µg OB, or (4) oil vehicle alone. These doses were chosen because they restore full sexual behaviour in the castrated male rat when OB and DHTP are given in combination^{4,5}, but not when each is administered alone²⁻⁵. Treatments were administered as two daily 0.1-ml subcutaneous injections, for 18-22 d. The hormones were provided for us in unlabelled, coded bottles, and we did not know which animal received which treatment until the experiment was finished and the code was broken.

For electrophysiological recording the animals were anaesthetized with urethane (1.3 g per kg, intraperitoneally)

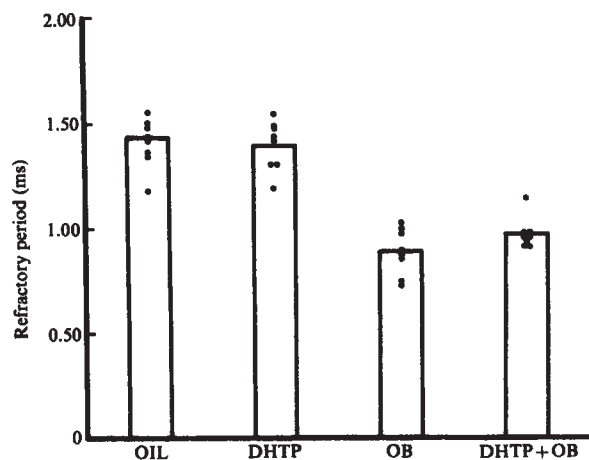


Fig. 1 Absolute refractory period of CMA neurones projecting to the MPH in castrated rats treated with oil (69 neurones from eight rats); DHTP (74 neurones from eight rats); OB (80 neurones from eight rats), or DHTP+OB (87 neurones from eight rats). Figure gives the overall mean and individual means (dots).

and mounted in a conventional stereotaxic frame (Kopf Instruments). Coordinates for electrode placement were the same as given in refs 6 and 7. A monopolar stainless steel electrode, insulated to within 0.5 mm of the tip, was used to stimulate the medial preoptic-anterior hypothalamic area (MPH) with cathodic monophasic pulses (current 50–500 μ A; duration 0.5 ms). Glass micropipettes filled with 0.5 M sodium acetate plus 2% pontamine blue were used to make single-unit extracellular recordings from CMA neurones. No recording electrode was used in more than one animal. At the end of each experiment a small current was passed through the stimulating and recording electrodes to facilitate histological localization. The rats were perfused *in situ* with a 10% formal saline to which a small amount of potassium ferrocyanide had been added. Brains were mounted in paraffin wax, sectioned at 15 μ m and stained in cresyl violet and luxol fast blue.

Absolute refractory periods were determined in a total of 310 CMA neurones stimulated antidromically from the MPH. The criteria for antidromic invasion and the method for estimating the absolute refractory period of CMA neurones were the same as in refs 6 and 7. The results were analysed as follows. The refractory periods for all neurones isolated in a given rat were reduced to a single mean. These means (eight for each of the four groups) were then analysed by analysis of variance, followed by the Scheffé test⁸.

Results are shown in Fig. 1. Overall analysis of variance showed that the groups were significantly different ($F=56.80$, with d.f., 3,28; $P<0.001$). The Scheffé test showed that oestradiol significantly reduced the refractory period (oil compared with OB, $P<0.001$). Dihydrotestosterone (unlike testosterone^{6,7}) had no effect when given alone (oil compared with DHTP was not significant) and no additional effect when given in combination with oestradiol (OB compared with DHTP+OB, not significant.)

The mean absolute refractory period for neurones in the group of gonadally intact rats in our earlier experiment was 1.01 ms, with 95% confidence limits of 0.89 and 1.13 ms; the mean for the group of castrated rats was 1.61 ms with 95% confidence limits of 1.37 and 1.85 ms. The mean absolute refractory periods for the OB and the DHTP+OB groups in this experiment (0.90 and 0.98 ms) are therefore within the 95% confidence limits of our earlier intact group and those for the group treated with DHTP (1.40 ms) are within the 95% confidence limits of our earlier castrated group. These comparisons demonstrate that the refractory periods for CMA-MPH neurones in animals that are castrated but treated with oestradiol are not significantly different from those in gonadally intact animals.

Thus, our results show that oestradiol has the same effect on a population of testosterone-sensitive forebrain neurones as testosterone itself, as would be predicted from much indirect evidence. Dihydrotestosterone, on the other hand, has no effect on these neurones, and does not enhance the effect that oestradiol has on them.

Although we have not excluded the possibility that higher doses of dihydrotestosterone might have such an action, these results suggest that in behavioural experiments with the two hormones in doses comparable to our own, the increased sexual behaviour that is induced by dihydrotestosterone is not attributable to an action of the hormone on forebrain neurones comparable with that of testosterone and oestradiol. There is independent evidence that dihydrotestosterone has a different site of action, for the stimulating effect of testosterone on spinal sexual reflexes is mimicked by dihydrotestosterone, but not by oestradiol⁹.

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Activity-dependent extracellular K^+ fluctuations in canine Purkinje fibres

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Cardiac Purkinje fibres are important in cardiac electrophysiology and pathology due to their specialized role in the ventricular conducting system, and their properties as a second cardiac pacemaker¹. Changes in extracellular K^+ activity (K_o) affect the conduction velocity² and the natural automatic rate of the Purkinje fibre³. Both anatomical⁴ and electrophysiological^{5,6} studies of the Purkinje fibres have demonstrated the possibility of K_o fluctuations in the narrow intercellular clefts. We investigated, using K^+ selective electrodes, the effect of the K_o fluctuations observed during activity in canine Purkinje fibres. Summation of K_o fluctuations during single action potentials leads to elevation of baseline K_o during rapid trains. However, unlike in ventricular muscle where depolarization is seen^{7,8}, membrane potential in non-automatic Purkinje fibres hyperpolarizes in response to rapid beating. Prolonged depletions of extracellular K^+ following long periods of overdrive are associated with slowly changing membrane currents, which markedly influence automaticity, action potential duration, and membrane potential.

Free running canine Purkinje fibres were removed from left and right ventricles and perfused in well oxygenated Tyrode solutions containing the following, 4 mM KCl, 2 mM $CaCl_2$, 130 mM NaCl, 1 mM $MgCl_2$, 0.4 mM NaH_2PO_4 , 12 mM $NaHCO_3$, and 6 mM glucose. pH was maintained at between

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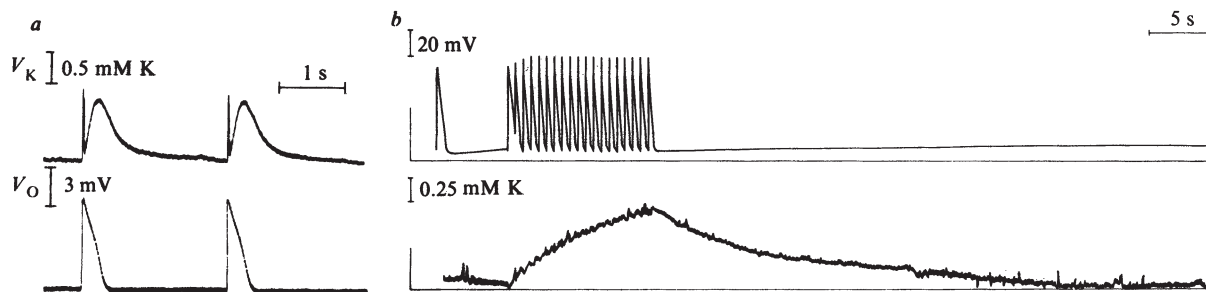


Fig. 1 (a), Top trace. (V_k) is the differential output of the two K^+ electrode barrels and represents the K_o fluctuations during two single beats. The initial spike on this trace is an artefact due to the inability to reject stimulus voltage. Bottom trace: (V_o) is simultaneous output of the K^+ electrode reference barrel (37°C). (b), Top trace. (V_m) is membrane potential measured with a conventional intracellular microelectrode. Bottom trace: (V_k) is differential output of K^+ electrode and represents K_o . No beat to beat accumulations are seen with this electrode tip position. K_o accumulates slowly during 10s of stimulation at 1 beat per 600ms (37°C).

1.2 and 7.4; the temperature was kept stable at the levels indicated.

Double barrel K^+ sensitive electrodes (Corning liquid ion exchanger 477317) were constructed from theta glass double channel tubing and calibrated for selectivity and response time using methods previously described⁷. To measure K^+ activity levels, the output of the Tyrode-filled reference barrel (V_o) was subtracted from the output of the K^+ selective barrel ($V_k + V_o$). The differential channel output (V_k) represents the K^+ activity levels, which for small changes vary approximately linearly with K^+ activity.⁸

Due to the somewhat slower response time of the K^+ selective barrel (20ms), capacitive compensation was used to speed this barrel while a variable 'roll off' filter (Bloom) was used to slow the reference barrel output before differential amplification. Common mode rejection of the two barrels was adjusted to within 0.1% by a variable gain circuit.

K^+ electrode experiments were performed on the largest of the canine Purkinje fibres to facilitate manipulation of the electrode and minimize the effect of tissue dimpling. Voltage clamp experiments were performed using the two-microelectrode voltage clamp method⁶ on the short fibres of arrow radius (length, 1.5mm; radius 0.15mm). These fibres were dissected into short sections while being continually perfused with a high calcium (4mM) Tyrode solution. In these small fibres, clamp steps could be applied with acceptable levels of current magnitude and a good spatial homogeneity⁹. These fibres have K^+ currents similar to those described in *in situ*¹⁰.

To measure K_o fluctuations, K^+ electrodes were slowly inserted through the sheath of the fibre bundle under constant observation through a dissecting microscope. To minimize tissue distortion, electrodes were made with fine tips (1–2 μm in total outer diameter). In fact, frequently cells were inadvertently punctured while the K^+ electrode was being advanced, such that normal action potentials were measured with the reference barrel, along with very high K^+ activity levels (but no beat to beat fluctuations) on the differential output. On withdrawal (or further advance) of the electrode tip, there was an abrupt fall in measured K^+ levels. At the same time, potential on the reference barrel returned to within several millivolts of ground level. One criterion for determining that the K^+ electrode was located extracellularly was that measured K^+ activity levels during quiescent equilibrium states were near bath levels, and were responsive to changes in bath K^+ levels⁸.

Figure 1a shows K_o accumulation measured during a single action potential. This beat to beat fluctuation occurs only when the electrode is near a cell membrane. The accumulation developed rapidly during the plateau of the action potential and then began to subside during fast repolarization. There is an initial rapid fall of K_o which may have been influenced by the effect of active uptake processes on the narrow cleft spaces. During rapid beating, in addition to the K_o fluctuations during single beats, there is an overall accumulation as beats occur progressively earlier during diastolic decay of K_o (ref. 8). The post train decay of K_o now exhibits a slow tail following the initial rapid decline, where the time constant of the slow

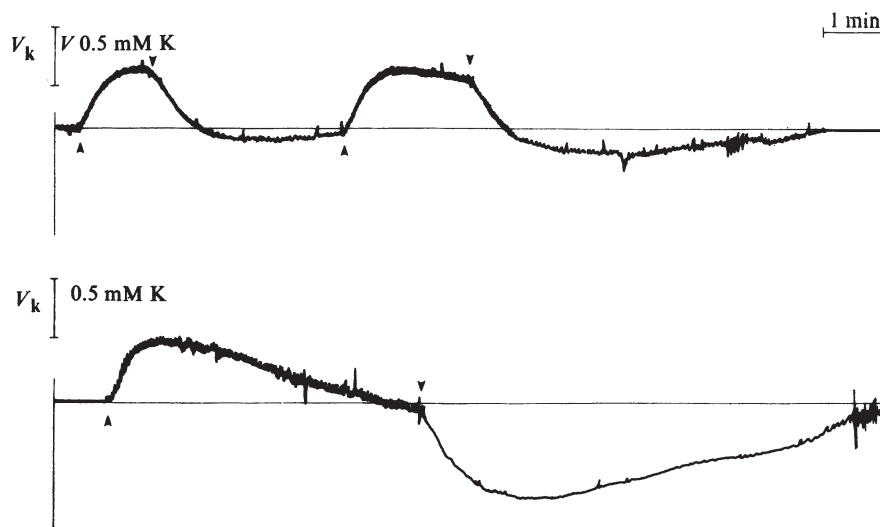


Fig. 2 K_o fluctuations (V_k) during and after 1, 2 and 5 min periods of overdrive at 1 beat per 300ms. K_o undershoot of the baseline implies extracellular K^+ depletion. Solid arrow 'up' indicates start of stimulus drive; arrow 'down' indicates stimulus drive off (37°C).

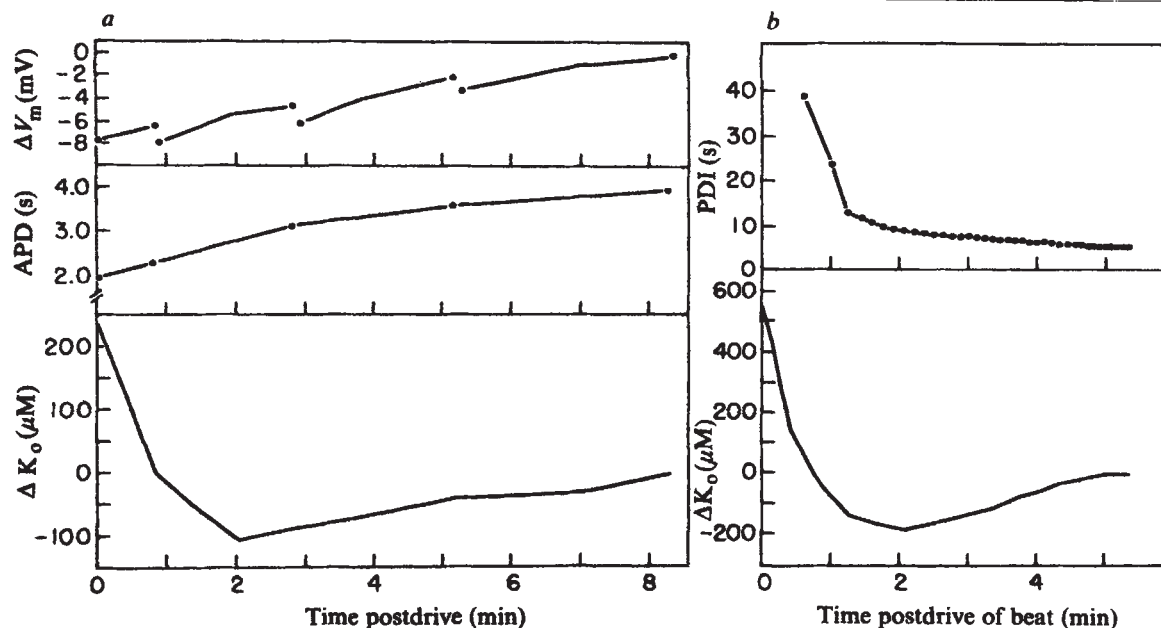


Fig. 3 (a), Return of V_m and action potential duration (APD) to control values following overdrive, plotted with simultaneously measured K^+ depletion. The '0' value on the K_0 axis corresponds to baseline levels of K_0 (22°C). (b), Comparison of return of automaticity with K_0 postdrive. Each point in the top panel represents a single action potential. Vertical axis is duration of previous diastolic interval (PDI) for each beat. (37°C; difference in time course of undershoot in a and b is due to temperature difference.)

component reflects the diffusion relaxation time for the entire cylindrical preparation⁷.

If the electrode tip is in a more superficial position in the fibre bundle, beat to beat accumulations are not seen. This is probably due to the longer diffusion pathway between the clefts and the electrode tip, and the resulting time average of observed K_0 levels⁷. As shown in Fig. 1b, K_0 accumulates progressively during 1 min of beating. We also measured intracellular potential (V_m) with a nearby conventional microelectrode, and found that the membrane hyperpolarizes during the K_0 accumulation.

With overdrive of more than 1 min, K_0 levels plateau and later slowly subside back to baseline. The most likely reasons for the decline in K_0 are stimulation of the Na^+-K^+ pump due to intracellular Na^+ accumulation^{11,12} and decreased K^+ efflux per beat, due in part to action potential shortening

(unpublished observation). When beating is terminated, K_0 levels fall rapidly and undershoot the baseline for a prolonged period. This prolonged postdrive undershoot is evidence of persistent Na^+-K^+ pump activity¹¹. Figure 2 shows such K_0 accumulation and depletion associated with rapid stimulus trains of varying length (1, 2 and 5 min). The magnitude and duration of the K_0 undershoot increases with longer periods of overdrive¹¹. Cooling the preparation to 22°C also prolonged the undershoot.

With an intracellular microelectrode in a nearby cell, we monitored membrane potential (V_m) simultaneously with K_0 values. On cessation of stimulation following a period of overdrive, membrane potential is hyperpolarized and automaticity is suppressed. This phenomenon of overdrive suppression was originally described by Vassalle¹³. In addition, action potential duration is shortened following

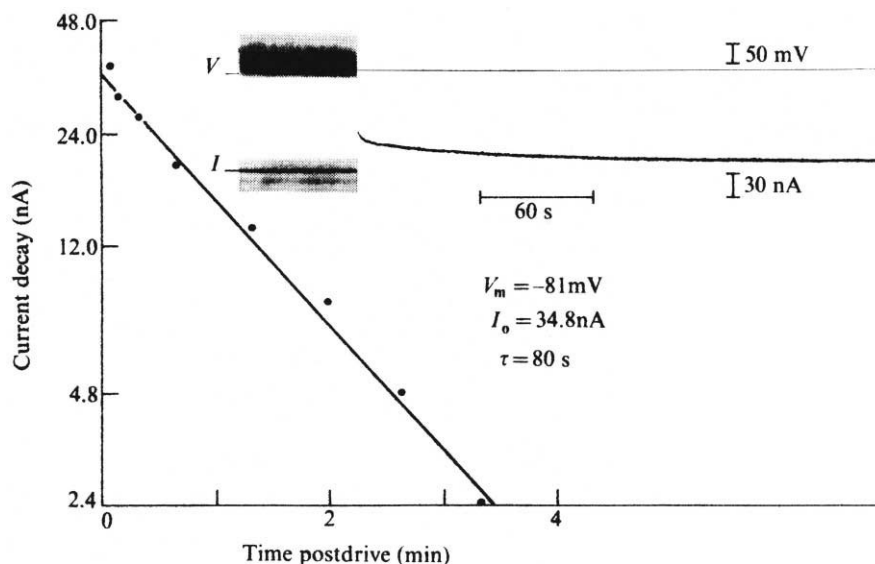


Fig. 4 Clamp current following overdrive (inset) is shown for 4 min voltage clamp to -81 mV (V, voltage trace; I, current trace).

drive. Figure 3a plots the postdrive return of action potential duration and membrane potential following a period of overdrive. Figure 3b plots the postdrive return of automatic activity versus K_0 for a spontaneously rhythmic fibre. The effect of overdrive on the membrane electrophysiological parameters gradually subsided during a period of minutes, and closely coincided with the duration of the K_0 undershoot.

As the post overdrive variations in these three parameters can be explained by a decaying outward current^{13,14}, we attempted to measure that current using the voltage clamp^{15,16}. Following a similar period of rapid drive, we measured currents using sustained voltage clamp pulses at various holding potentials. Figure 4 (inset) shows the current recorded during a 4-min clamp pulse to -81 mV, following a period of overdrive. A decaying outward current is readily seen. The time constant of the current is 80 s, 1–2 orders of magnitude larger than the time constant of the pacemaker current (I_{K2}) measured at this holding potential^{10,17}. This slow current is dependent on there being a preceding period of overdrive, and is enhanced as the drive duration is lengthened.

However, the current does not seem to be independent of potential. As the postdrive clamp potential becomes more positive, the magnitude of the current decreases. Between -50 and -40 mV, the current becomes biphasic, and eventually at more positive potentials reverses polarity. The time constant of the decay of the slow current also seems to vary with potential. This may arise from: (1) contamination of an electrogenic pump current due to K_0 accumulation or depletion acting on other currents; (2) contamination of an electrogenic pump current by another time- and voltage-dependent current (I_x); (3) a direct effect on pump current of changing levels of K_0 in the clefts; (4) a dramatic dependence of passive ionic fluxes on post drive clamp potential, causing, for example, an increase in sodium loading at depolarized potentials; or, (5) a complicated dependence of pump current on membrane potential. Further experiments are needed to determine why slow current decay varies in this way with potential.

In summary, there is direct evidence for beat to beat changes in extracellular K^+ activity in canine Purkinje fibres. These fluctuations may provide some of the time dependence of the K^+ currents in both the plateau and diastolic potential ranges, by altering both the K^+ equilibrium potential and K^+ conductance¹⁸. It is also possible that transient changes in K_0 during extra-systoles and short periods of triggered automaticity may influence the following beats. Therefore, these activity-dependent fluctuations in K_0 and the associated membrane currents may be important factors affecting both the generation and maintenance of some ventricular arrhythmias.

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Single acetylcholine-activated channels show burst-kinetics in presence of desensitizing concentrations of agonist

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High resolution measurements of the current through individual ion channels activated by acetylcholine (AChR-channels) in frog muscle have shown that these currents are discrete pulse-like events with durations of a few milliseconds^{1,2}. Fluctuation and relaxation measurements of end-plate currents have led to the conclusion that the rate of channel opening increases with agonist concentration, and that the channel, once open, closes spontaneously^{3,6}. Katz and Thesleff have shown, however, that in the continued presence of ACh, the initial end-plate current declines to an equilibrium value with a time constant of several seconds⁷. This reversible phenomenon is referred to as receptor desensitization. We report here that in the presence of ACh concentrations sufficient to cause desensitization, single channel current pulses appear in groups. From the temporal sequence of the pulses, we have derived estimates of the rates of activation and desensitization of the AChR-channel.

Experiments were performed on the extra-synaptic membrane of denervated fibres of frog (*Rana pipiens*) cutaneous pectoris muscle, using the extra-cellular patch clamp method for the application of agonist and to record membrane current⁸. AChR-channels in the extra-synaptic membrane are heterogeneous in their mean 'open times' (E.N. and B.S., in preparation). The predominant extra-synaptic AChR-channel has been described as having open times four to five times longer than the synaptic-type channel. However, about 10% of the channels we observed had properties comparable to subsynaptic AChR-channels. This report includes only measurements from channels of this latter type to facilitate comparison with previous measurements of end-plate currents.

Figure 1a shows the current recorded from a patch of membrane in the presence of $5 \mu\text{M}$ ACh starting less than 1 s after the agonist-containing pipette had been sealed on to the muscle fibre. The current switched randomly between the resting level and four equally spaced amplitudes, indicating that at least four channels were active. The progressive decrease in the number of amplitudes reached in the continued presence of ACh indicated a decrease in the number of open channels. This decrease characterizes the time course of receptor desensitization and results in complete inactivation of the current because of the low density of AChR-channels in the extrasynaptic membrane. However, after this initial desensitization, 'bursts' of single channel currents are observed at irregular intervals. Figure 1b shows a sequence of four such bursts recorded in the presence of $10 \mu\text{M}$ ACh about 30 s after the pipette had been sealed against the membrane. Initially the membrane is at the resting level. At time A, the current begins to fluctuate rapidly between the resting and another discrete level which in its amplitude corresponds to that flowing through a single open channel. At time B, about 800 ms later, the membrane current returns to the resting level. These rapid fluctuations define a current burst which is followed by an interburst interval during which the membrane is at the resting level. The sequence of bursts and interburst intervals becomes apparent in the presence of ACh concentrations $> 2 \mu\text{M}$.

We interpret this pattern of current to reflect fluctuations of single AChR-channels between three states: closed, open and a desensitized, non-conducting state. At the beginning of the record shown in Fig. 1b, according to this model, all AChR-channels of the membrane patch under investigation are in a

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desensitized state. At time A, a single receptor-channel complex converts from the desensitized to the open/closed state. It then fluctuates rapidly between the open and the closed state and converts again to the desensitized state at B. However, the pattern of current shown in Fig. 1b is also reminiscent of the behaviour of single AChR-channels in the presence of channel-blocking substances⁹. Because some agonists themselves can block channels, the observed fluctuations of current during a burst could also reflect blocking of open channels by ACh molecules¹⁰. The two alternative mechanisms can be distinguished by their predictions for the agonist concentration dependence of the kinetics of fluctuations of current within a burst.

In the first interpretation, the rapid fluctuations are due to the changes of state: closed $\xrightleftharpoons[\beta']{\alpha}$ open. In this two-state scheme β' and α are the rate constants of channel opening and closing respectively: α should be independent of the ACh concentration and can be measured as the reciprocal decay constant, τ_o^{-1} , of the distribution of the durations of current pulses (mean 'open time') during a burst. On the other hand, β' , measured as the reciprocal decay constant, τ_c^{-1} , of the distribution of intervals between pulses, (mean 'closed time') should increase with the ACh concentration, because β' is an apparent rate constant, whose value is assumed to depend on agonist-binding steps preceding channel openings.

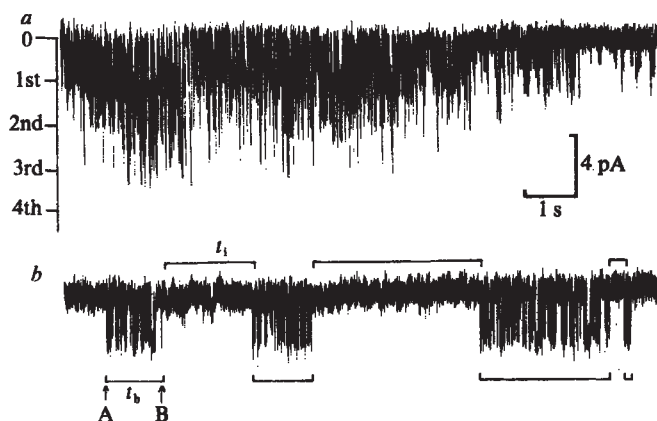


Fig. 1 Onset of desensitization and burst kinetics on small patches of extra-junctional membrane of chronically denervated frog muscle in the maintained presence of constant ACh concentrations. *a*, Record from a membrane patch starting when the agonist-containing patch pipette was sealed against the membrane. Four discrete levels of current can be resolved, as indicated roughly by the scale on the left. Individual step sizes are ≈ 3 pA, as expected for open ACh-activated channels at -130 mV membrane potential. The occurrence of currents reaching the fourth level indicates that at least four channels are active simultaneously. The maximum current amplitude reached decreases during this record until the membrane patch is at the resting level. ACh concentration was $5 \mu\text{M}$; membrane potential, -130 mV; temperature, 11°C . Inward current was downwards. The background noise, seen clearly on the resting level, caused some spread of the current amplitudes at each level. All records have been filtered at 500 Hz low pass. *b*, Bursts of current fluctuations follow the period of initial current inactivation. This record was started 34 s after the placement of the patch pipette and following a current pattern comparable to that shown in (*a*). The arrows marked A and B indicate the onset and cessation of fluctuations between the resting and the first current level and delineate that which is defined as a single channel current burst. Brackets indicate the measurement of t_b and t_i , burst duration and the inter-burst interval, respectively. Although occasionally bursts occur simultaneously, giving rise to currents reaching the second level (not illustrated), most bursts late in the record only reach the first level of current. This indicates that only a single channel is active. Conditions are the same as in (*a*), except that the pipette contained $10 \mu\text{M}$ ACh. The calibration scales apply to both traces.

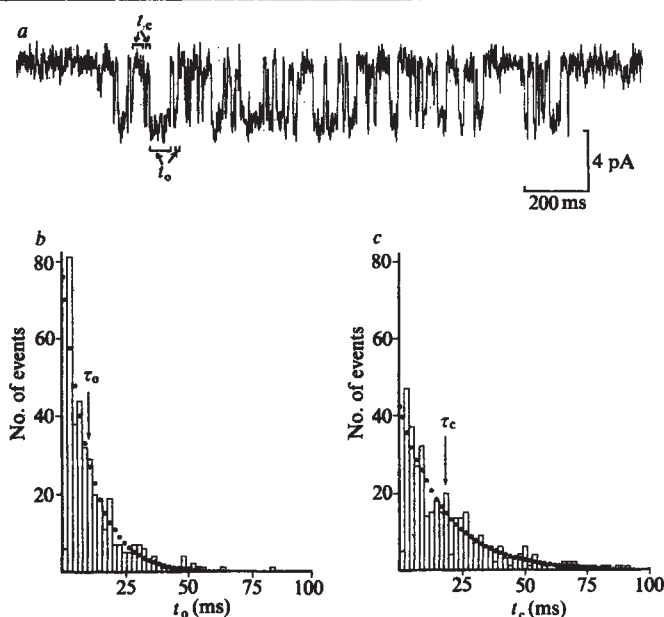


Fig. 2 Analysis of the rapid fluctuation of current during a burst. *a*, A single recorded burst in the presence of $10 \mu\text{M}$ ACh shown at higher time resolution than those in Fig. 1b. Brackets indicate the measurement of individual channel open times, t_o , and closed times, t_c . The membrane potential was -130 mV; temperature, 12°C . *b* and *c*, Histograms of distributions of open and closed times measured during several sequential bursts at $10 \mu\text{M}$ ACh, -130 mV membrane potential and 12°C . Records of currents were stored on an FM tape recorder, subsequently sampled and stored digitally on a PDP 11/34 computer. The sampling interval was 0.5 ms. Individual open and closed times were measured semi-automatically and stored to construct frequency-of-occurrence histograms. The histograms were fitted with single exponential curves, as indicated by the dotted line, using a least-squares fitting routine weighted for the statistical nature of the underlying events. The arrows indicate the best fit decay constants τ_o and τ_c for the distribution of open and closed times, respectively. τ_o was 10.6 ms and τ_c was 18 ms at $10 \mu\text{M}$ ACh.

In contrast, a channel-blocking mechanism would predict the opposite. The mean open time would shorten as the agonist concentration was raised, and the mean closed time would be independent.

We have, therefore, analysed quantitatively the distribution of open and closed times within bursts at various concentrations of ACh. Figure 2a shows a burst, illustrating the time course of the channel's conductance fluctuations. The distributions of open and closed times can be fitted by single exponentials (Fig. 2b, c), as expected for first-order kinetic processes. Figure 3a shows single bursts recorded in the presence of various concentrations of ACh and Fig. 3b summarizes our measurements as a plot of the values of τ_o^{-1} and τ_c^{-1} in double log scales as a function of ACh concentration. The mean open times are not measurably concentration dependent. Measurements at -90 mV and -130 mV show that mean open times increase with increasing membrane hyperpolarization. The e-fold increase per 65 -mV change is close to the value reported for single channel measurements at low ACh concentration¹. The rate constants of channel closing derived from these values are of the order of 100 – 250 s^{-1} , comparable to the values inferred from end-plate current fluctuation analysis⁴. Channel closed times decrease with agonist concentration. In the range of 5 – $50 \mu\text{M}$ ACh, the relation between τ_c^{-1} and ACh concentration is roughly described by a power law with an exponent of about 1.4 . This suggests an increase of the apparent rate constant of channel opening from $\sim 25 \text{ s}^{-1}$ to $\sim 500 \text{ s}^{-1}$ in this concentration range. It is consistent with a reaction scheme that involves

the binding of two ACh molecules preceding the opening of a single channel^{5,6}. The intersections between the lines in Fig. 3b define the concentration of ACh for which the mean open time equals the mean closed time. These concentrations, 22 μM at -90mV and 17 μM at -130mV , are estimates of the apparent dissociation constants for channel activation by ACh in our conditions. These results support the hypothesis that the rapid current fluctuations during a burst reflect transitions of a single channel between the open and closed states. They rule out a channel-blocking mechanism.

The repeated appearance and disappearance of bursts of current, as illustrated in Fig. 1b, reflects fluctuations of AChR-channels between the open/closed and a desensitized state occurring on a much slower time scale than the open-closed transitions of the channel. At even lower time resolution, however, bursts seem to be an element of a pattern of current to which we refer as 'clusters' of several bursts (Fig. 4). Clusters are evident because the interburst intervals are much shorter than the mean intervals between the clusters.

Bursts and interburst intervals within a cluster vary stochastically in duration, and bursts become shorter at higher ACh concentrations. The separation of bursts is not always seen as clearly as in Fig. 1b, and in some cases it is difficult to define the beginning and end of individual bursts. However, at 20 μM ACh, bursts are clearly resolved (Fig. 4). The mean values of burst durations and interburst intervals at this concentration are $420 \pm 12\text{ms}$ and $180 \pm 10\text{ms}$ ($\pm\text{s.e.}$), respectively. Clusters of bursts have durations of several seconds and are separated by irregular intervals of several tens of seconds. For example, the cluster of eight bursts illustrated in Fig. 4 was recorded 18 s after the pipette had touched the muscle membrane; the next cluster occurred 23 s later. The

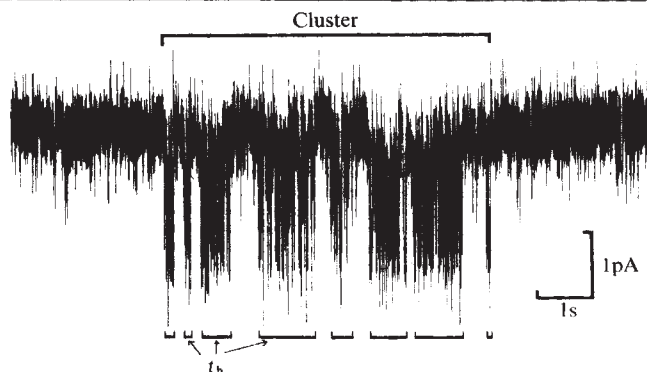


Fig. 4 Occurrence of single channel bursts in clusters as revealed at low time resolution. This record was obtained in the presence of 20 μM ACh 18 s after the establishment of the pipette-membrane seal. Membrane potential was -90mV , 12°C . At this slow sweep speed it is no longer possible to resolve individual pulses of current, but a clear grouping of bursts of pulses is apparent. The lower brackets indicate the durations of eight individual bursts, while the upper bracket illustrates the duration of a cluster. In frequency-of-occurrence histograms of the interval between current pulses, three widely separated exponential components are measurable. The fastest component represents the distribution of channel closed times within bursts, the intermediate one the distribution of inter-burst intervals and the slowest component represents the time intervals between clusters. The wide separation of the means of the various interval distributions justifies the separation of groups of pulses into bursts and clusters.

duration of clusters is variable and decreases with increasing agonist concentrations. At 20 μM ACh, the average duration of clusters is $4.8 \pm 2\text{s}$, whereas the average duration of the interval between clusters is $34 \pm 4\text{s}$. A simple explanation for these observations is that the AChR-channel can exist in two different states of desensitization. Thus at 20 μM ACh, the sequence of bursts and burst intervals reflects the kinetics of a fast component of desensitization with apparent rate constants of $\approx 2\text{s}^{-1}$ and $\approx 5\text{s}^{-1}$ for the conversion to and from the desensitized state, respectively. The kinetics of a slow component are measured by the average duration of clusters and the average interval between clusters. At 20 μM ACh, the apparent rate constants are $\approx 0.2\text{s}^{-1}$ and $\leq 0.03\text{s}^{-1}$ for the transitions between open/closed and this desensitized state.

The results demonstrate that at least three kinetic processes control the AChR-channel conductance state in the presence of desensitizing concentrations of ACh. The rapid fluctuations of current in the millisecond time range represent transitions of single AChR-channels between their open and closed states. The slow kinetic process, in the time range of several seconds, which is measured as the repeated occurrence of clusters, can tentatively be related to the onset of and recovery from desensitization as observed in measurements of end-plate current¹¹⁻¹³. In addition, we have presented kinetic evidence for an agonist-receptor interaction that leads to a desensitized state of the receptor-channel complex in the intermediate time range of hundreds of milliseconds.

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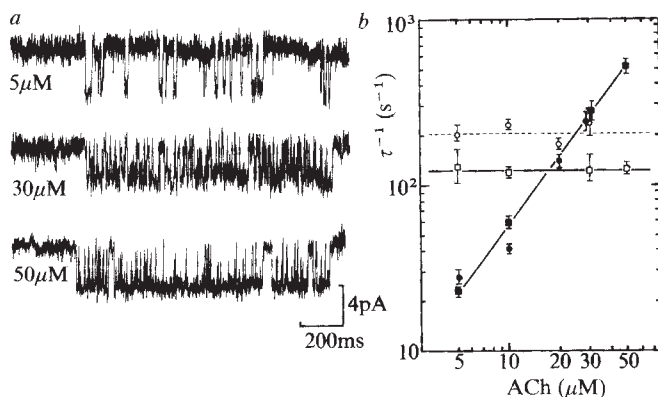


Fig. 3 Agonist concentration dependence of open and closed times during current bursts. *a*, Examples of bursts recorded in the presence of three different ACh concentrations as indicated on the left side of each record. All three records were taken at -130mV membrane potential, 11°C . As the ACh concentration increases, the duration of intervals between pulses of current decreases. At ACh concentrations $<20\mu\text{M}$ ACh, the channels are, during a burst, mostly in the closed state, whereas at $>20\mu\text{M}$ ACh, the channels are predominantly in the open state. *b*, Log log plot of the inverse of the best fit τ_0 and τ_c values, measured as shown in Fig. 2b and c against ACh concentration. Open symbols are values for the channel mean open times, τ_0 , and closed symbols are for the channel mean closed times, τ_c . Circles were measurements at -90mV , squares at -130mV membrane potential. The solid lines were drawn through the -130mV data, the dotted through the -90mV . All lines were drawn by eye. Each point represents the best fit exponential to histograms made by summation of the histograms from many bursts in the same conditions. Standard error brackets refer to the calculated standard error of the best fit values of τ to their respective histograms. Channel open times are not measurably dependent on ACh concentration but increase with membrane hyperpolarization. The channel closed times are strongly dependent on ACh concentration; the slope of the line drawn was 1.4. No consistent change of mean closed times with changes in membrane potential was measurable.

Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats

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Since the demonstration of dopamine-containing nerve terminals within cortical regions^{1,2}, studies have confirmed the existence of a dopaminergic projection to the medial prefrontal cortex^{3,4}. Cortical dopamine (DA) may be a functional neurotransmitter, as there are selective high-affinity receptor binding^{5,6} and uptake sites^{7,8} within this area, a DA-selective adenylate cyclase system^{9,10}, cells responsive to iontophoretically applied DA¹⁰, and a pool of DA amenable to release *in vivo*¹¹ or *in vitro*^{12,13}. Lesions of either midbrain cell bodies in the ventral tegmental area^{14,15} or of frontal cortical areas directly¹⁶, with subsequent loss of DA terminals from the medial prefrontal cortex, induce hyperactivity in rats¹⁶, and enhanced behavioural responses to amphetamine^{16,17}. Lesion of subcortical DA pathways results in the opposite effects, suggesting that frontal cortical DA systems may have an inhibitory role in motor behaviour^{15,16}. Dopamine-dependent hyperactivity and stereotypy is believed to be mediated from extrapyramidal and mesolimbic DA-innervated sites¹⁸. We previously noted that loss of catecholamine terminals within the frontal cortex of the rat results in increased DA turnover and utilization in subcortical striatal and limbic regions after one week¹⁹. We now show evidence of both enhanced pre-synaptic and post-synaptic mechanisms in the terminal regions of the ascending nigrostriatal and mesolimbic DA pathways one month after destruction of DA terminals within the medial prefrontal cortex of the rat. The increased ³H-ADTN and ³H-spiperone binding, enhanced DA-induced stimulation of adenylate cyclase and potentiated uptake capacity for DA within striatum and nucleus accumbens may demonstrate a functional basis for the observed potentiated behavioural responses.

Destruction of catecholamine terminals within the area of the medial prefrontal cortex was performed by infusion of 6-hydroxydopamine (6-OHDA) into this site. Male Porton rats (220–250 g) were pretreated with the noradrenergic uptake blocker desmethylimipramine²⁰ (DMI, 25 mg per kg, intraperitoneally (i.p.), 30 min before lesion) to increase the relative toxic specificity of 6-OHDA for DA terminals. Animals were anaesthetized (chloral hydrate, 300 mg per kg), and immobilized in a Kopf stereotaxic frame. Two groups of rats received bilateral injections of either solvent (1.5 µl 0.1% nitrogen-bubbled sodium metabisulphite solution) or 6-OHDA (Sigma) (8 µg per 1.5 µl per 2 min) into the medial prefrontal cortex (coordinates A + 10.3; V + 1.0; L ± 0.8 mm)²¹ (Fig. 1).

Four weeks after surgery, the spontaneous and (+)-amphetamine (1 mg per kg, i.p.)-induced locomotor activity of both sham and lesioned rats was tested in an open field. Remaining rats were killed for biochemical assessment of the effects of the 6-OHDA/DMI lesion. Paired corpus striatum, nucleus accumbens and the region of the medial prefrontal cortex were rapidly dissected out and regional concentrations of DA and noradrenaline (NA) were measured using a sensitive radiometric enzymatic assay with [³H]-S-adenosyl-methionine (15 Ci mmol⁻¹; Radiochemical Centre, Amersham) as the methyl donor²². The high-affinity benzotropine-resistant²³ uptake of NA, and the DMI-resistant uptake of DA, within the lesion site and adjacent cortical areas were also compared (see Fig. 1). In addition, the DMI-resistant uptake of DA in the striatum and nucleus accumbens was measured in the two animal groups. Concentrations of the major DA metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) within striatum and nucleus accumbens were measured fluorimetrically²⁴, and the basal and dopamine (5–50 µM)-stimulated adenylate cyclase activity assayed in these regions according to the technique of Clement-Cormier and coworkers²⁵. Further, (+)-butaclamol-sensitive ³H-spiperone (20 Ci mmol⁻¹; Radiochemical Centre, Amersham) binding using a range of 100–2,000 pM radioactive ligand²⁷, and also the 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN)-sensitive binding of ³H-ADTN (4.9 Ci mmol⁻¹; Radiochemical Centre, Amersham) using a range of 5–40 nM radioactive ligand²⁸ were studied in striatal

Regional catecholamine uptake (fmol per mg tissue per min)

area:	1	2	3	Cg
³ H-dopamine	331 ± 39	165 ± 11	85 ± 4	330 ± 38
³ H-noradrenaline	357 ± 15	320 ± 10	310 ± 26	392 ± 24

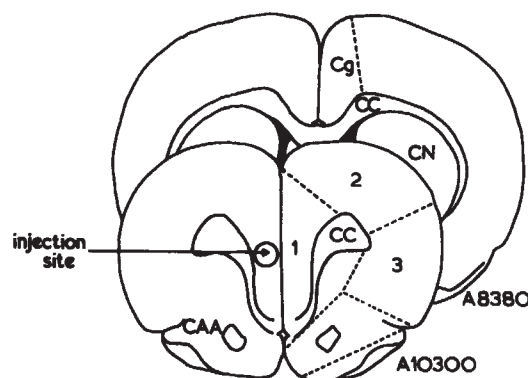
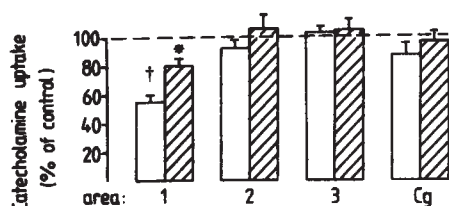


Fig. 1 The site and effect of 6-OHDA lesions of the medial prefrontal cortex on regional frontal cortical high-affinity catecholamine uptake. Transverse sections of the rat brain, levels A8380 and A10300²¹, indicate site of injection (bilaterally, area 1) and adjacent areas taken for regional assay (areas 2, 3 and Cg). For regional high-affinity catecholamine uptake, areas were dissected from the appropriate plane and tissue from both sides of each animal pooled and chopped in two directions at 0.2 mm intervals using a Mcllwain tissue chopper. Slices were weighed (5–10 mg per tube) incubated at 37 °C in 2 ml oxygen-bubbled Krebs–Ringer bicarbonate buffer, pH 7.4 for 10 min. ³H-DA (specific activity 5 Ci mmol⁻¹, Radiochemical Centre, Amersham) or ³H-NA (specific activity 10.7 Ci mmol⁻¹, Radiochemical Centre, Amersham) were added to each tube to give a final concentration of 0.1 µM. For DA uptake, 0.5 µM DMI was present in the buffer to restrict any uptake into NA terminals²⁰; for NA uptake, 1 µM benzotropine was present to restrict any uptake into DA terminals²³. Uptake was terminated after 5 min by rapid filtration on to Whatman GF/B filters, and, following washing with 15 ml ice-cold buffer, radioactivity was determined by liquid scintillation counting. Tissue and filter blanks were assessed by measuring the uptake of radioactivity at 0 °C. The results of the regional high-affinity catecholamine uptake were calculated as fmoles per mg tissue per min (upper trace). Uptake in 6-OHDA lesioned animals was expressed as a percentage of that obtained in sham-operated rats (lower trace); open columns represent DA uptake, hatched columns for NA uptake. Each point is the mean of six values; vertical bars denote standard errors. Cg, cingulate cortex; CN, caudate nucleus; CC, corpus callosum; CAA, anterior commissure.

**P* < 0.05; †*P* < 0.01 (Student's *t* test).

Table 1 Changes in regional concentrations of noradrenaline and dopamine and its metabolites in cortical and subcortical sites in rats following 6-OHDA lesions of the medial prefrontal cortex

Substance tested	Medial prefrontal cortex		Striatum		Nucleus accumbens	
	Sham	Lesion	Sham	Lesion	Sham	Lesion
NA	0.84 ± 0.03	0.58 ± 0.12* (68)	—	—	—	—
DA	0.11 ± 0.02	0.04 ± 0.01† (38)	11.94 ± 0.87	12.61 ± 0.84 (106)	7.97 ± 0.85	9.35 ± 0.40* (117)
HVA	—	—	0.63 ± 0.06	0.77 ± 0.04* (124)	0.93 ± 0.08	1.31 ± 0.10† (141)
DOPAC	—	—	1.21 ± 0.09	1.87 ± 0.40† (155)	1.33 ± 0.14	1.94 ± 0.27* (146)

Lesioned animals were pretreated with DMI (25 mg per kg, i.p.) and 6-OHDA (8 µg in 1.5 µl) injected bilaterally into the medial prefrontal cortex. Sham animals received bilateral injections of vehicle (1.5 µl 0.1% sodium metabisulphite solution) into the same site. Biochemical assays were conducted 30 days after surgery. Results are the mean of seven observations and are expressed as µg per g wet weight tissue ± 1 s.e.m. Figures in parentheses indicate the per cent value of sham-operated concentrations.

* $P < 0.05$.

† $P < 0.01$ (Student's *t* test).

and nucleus accumbens membrane preparations.

Total specific binding of ^3H -ADTN and ^3H -spiperone was significantly increased in the membrane fraction prepared from both striatum and nucleus accumbens examined from the 6-OHDA lesioned animals (Fig. 2). Scatchard analysis of the data indicated that this was due to an increase in receptor numbers in both regions for both ligands (ADTN: B-values for striatum, control 113 ± 10 fmol per mg protein compared to 146 ± 9 for lesioned group ($P < 0.05$); nucleus accumbens, control 78 ± 4 fmol per mg protein compared to 90 ± 5 for lesioned group ($P < 0.05$). Spiperone: B-values for striatum, control 0.81 ± 0.07 pmol per mg protein compared to 1.08 ± 0.09 for lesioned group ($P < 0.05$); nucleus accumbens, control 0.91 ± 0.05 pmol per mg protein compared to 1.33 ± 0.11 for lesioned group ($P < 0.05$). Additionally, there appeared to be a small decrease in receptor affinity for ^3H -ADTN binding in the striatum (K_d values: control 0.87 nM, lesion 1.54 nM). There appeared to be no change in receptor affinity with dissociation constants ranging from 0.22 to 0.49 nM for ^3H -spiperone binding, or for ^3H -ADTN binding in the nucleus accumbens, the regression analysis showing that the slopes did not significantly deviate from parallelism between sham-operated and lesioned animals. All binding data represents mean ± s.e.m. of eight determinations.

Dopamine (5–50 µM) markedly stimulated adenylate cyclase activity in striatal and accumbens homogenates. This effect was significantly greater in 6-OHDA lesioned animals at all doses of DA giving maximal stimulation in the striatum ($P < 0.05$) and at the 20 µM dose of DA in the nucleus accumbens ($P < 0.05$, Fig. 3). Furthermore, the cortical lesions resulted in significant increase in the high affinity DMI-resistant uptake of DA in both extrapyramidal and mesolimbic sites. Accumulation of DA into striatal slices from 6-OHDA/DMI lesioned rats was $1,067 \pm 71$ fmol per mg tissue per min compared to 754 ± 70 fmol per mg tissue per min in vehicle-injected animals ($2P < 0.02$). Accumulation of DA into slices from nucleus accumbens was 649 ± 86 fmol per mg tissue per min in 6-OHDA/DMI lesioned rats: control animals accumulated 435 ± 32 fmol per mg tissue per min ($2P < 0.02$, $n = 10$).

We have previously reported the behavioural and biochemical sequelae of such lesions, 10 days after surgery^{16,19}. Because the additional binding and uptake studies were performed 1 month after surgery, we repeated these experiments at this later time to verify the effects of the lesion. At 1 month these lesions enhanced spontaneous and amphetamine (1 mg per kg, i.p.)-induced locomotor activity in the perimeter squares of an open field compared to sham-lesioned animals (spontaneous, $P < 0.05$; amphetamine-induced, $P < 0.01$, $n = 8$). Similarly the biochemical parameters at 1 month paralleled those results reported at 10 days after

surgery. Briefly 6-OHDA lesions of the medial prefrontal cortex reduced DA concentrations to 38% ($P < 0.01$) and NA concentrations to 68% ($P < 0.05$) within this site (Table 1). These results were supported by a 48% decrease in high-affinity DMI-resistant DA uptake and a 21% decrease in high-affinity benztrapine-resistant NA uptake (Fig. 1). These significant changes in catecholamine uptake were only observed in the region of the medial prefrontal cortex (area 1), adjacent regions and the cingulate cortex showed no differences between 6-OHDA lesioned and sham-operated animals (Fig. 1). In addition to changes in cortical catecholamine content, significant increases (+17%) in the DA content of the nucleus accumbens ($P < 0.05$), and HVA and DOPAC concentrations in both striatum and nucleus accumbens were recorded in 6-OHDA lesioned rats (Table 1).

Lesions of 6-OHDA of the medial prefrontal cortex produce significant loss of both DA and NA from this site, together with an apparent enhancement of subcortical DA systems as indicated by increases in concentrations of DA and its metabolites, in basal and dopamine-stimulated adenylate cyclase, in ^3H -ADTN and ^3H -spiperone receptor binding and enhanced high-affinity uptake of DA within the striatum and nucleus accumbens. The neurotoxic effects of the lesion seem to be confined to the medial prefrontal cortex as adjacent regions and the cingulate cortex showed no changes in high-affinity catecholamine uptake. The lesion was associated with an increase in both spontaneous and amphetamine-induced locomotor activity.

The biochemical data would suggest a facilitation of DA mechanisms within extrapyramidal and mesolimbic sites which would support the enhanced behavioural responses. This increase would seem to involve both pre- and postsynaptic mechanisms within the striatum and nucleus accumbens as indicated by the recorded increase in DA utilization (increased DA metabolite concentrations), DA uptake, and increase in adenylate cyclase and receptor number. The exact site of these receptor increases is not known as not all postsynaptic DA receptors seem to be linked to adenylate cyclase²⁹ and a recent report suggests only a poor correlation between ^3H -spiperone binding and DA-sensitive adenylate cyclase suggesting that two different populations of DA receptors may be involved³⁰. Furthermore it has been suggested that the neuroleptic may also label central serotonin receptors which would obviously confuse interpretation of the results³¹. However, an identical binding pattern is observed in lesioned animals using ADTN, a rigid analogue of DA which is believed to bind specifically to DNA receptors²⁸.

Such an apparent increase in both presynaptic and postsynaptic neurotransmitter mechanisms seem paradoxical as a decreased presynaptic function is usually associated with an increased postsynaptic sensitivity³² and vice versa³³. Thus

the appearance of increased activity at both pre- and postsynaptic sites is a novel situation which is very interesting because of its accordance with the post-mortem biochemical data seen in schizophrenia, where increases in DA concentrations in nucleus accumbens³⁴, and enhanced neuroleptic binding in both nucleus accumbens and striatum^{35,36} have been a documented feature of this disease. On the basis of other reported data, such a unilateral increase in pre- and postsynaptic activity is not completely without precedent, as recent findings describe increased behavioural responses following chronic treatment with DA agonists^{37,38}.

The mechanism by which loss of catecholamine terminals within the prefrontal cortex induces supersensitivity within subcortical DA pathways is not known. It may reflect an increased number of active DA terminals in these sites, but whether such changes are purely a physiological consequence of reducing cortical DA function, or related to collateral sprouting³⁹ following damage to the mesocortical DA projection remains to be resolved. In a previous study we showed that such lesions will also bring about changes in other neurotransmitter functions within the basal ganglia¹⁹. It therefore seems that the frontal cortical catecholamine system may be intimately concerned with the regulation of subcortical DA pathways. The medial prefrontal cortex sends

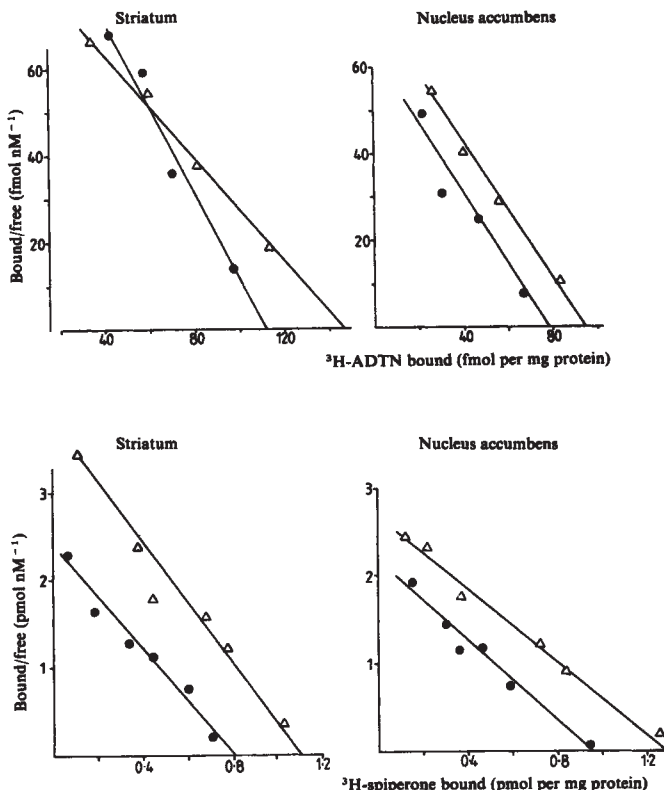


Fig. 2 Effect of 6-OHDA lesions of the medial prefrontal cortex on ³H-ADTN (upper traces) and ³H-spiperone (lower traces) binding to membrane fractions prepared from rat striatum and nucleus accumbens. Tissue pooled from nine sham-operated or nine 6-OHDA lesioned animals for each ligand, 30 days after surgery. Total specific ADTN binding was obtained by displacement with 1 μ M ADTN²⁸ and total specific ³H-spiperone binding was obtained by displacement with 1 μ M (+)-butaclamol²⁷. Data is shown as representative Scatchard plots of radiolabelled ligand receptor binding from sham-operated (●) and 6-OHDA lesioned animals (△), each point being the mean of quadruplicate determinations. At saturating concentrations of ³H-spiperone specific binding typically represented 500 c.p.m.; nonspecific being typically 200 c.p.m. At saturating concentrations of ADTN specific binding was typically 900 c.p.m.; nonspecific being 400 c.p.m.

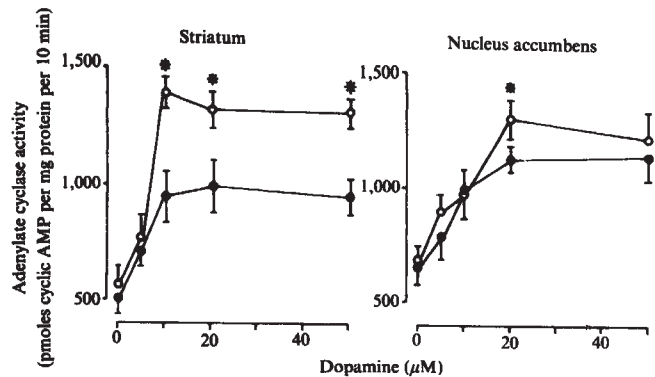


Fig. 3 Effect of 6-OHDA lesions of the medial prefrontal cortex on dopamine-sensitive adenylate cyclase activity in rat striatum and nucleus accumbens. Tissue pooled from six sham-operated or 6-OHDA lesioned rats, 30 days after surgery. DA-sensitive adenylate cyclase activity was determined according to the technique of Clement-Cormier *et al.*²⁵ and cAMP in the samples was assayed using the method of Brown *et al.*²⁶. The results shown represent activity in tissue from sham-operated animals (●) and 6-OHDA lesioned rats (○) and represents the mean $\pm$ 1 s.e.m. of three determinations each assayed in triplicate. The data shown is obtained from a single assay and is one of several experiments carried out in identical conditions and giving similar results. Significant differences from sham-treated animals ($P < 0.01$) are indicated by the asterisks and are determined non-parametrically from data pooled from two separate assays ($n = 6$).

projections to the striatum⁴⁰, nucleus accumbens⁴¹, ventral tegmental area⁴⁰, and to the substantia nigra⁴², thus providing potential routes through which such control may be mediated. The development of supersensitive central receptor sites is of much interest to the clinician, and an increase in either affinity or number of subcortical DA receptors has been suggested in development of Parkinson's disease⁴³, schizophrenia^{35,36} and the dyskinesias⁴⁴. Indeed a recent report has presented evidence for the development of supersensitive striatal DA receptors in rats following chronic neuroleptic treatment⁴⁵, a situation that may explain tardive dyskinesias which often develop in this type of drug therapy. That an apparent supersensitive subcortical DA system can also develop after lesions of the prefrontal cortex may have far reaching implications as to the possible involvement of this site in the aetiology and management of neurological and psychiatric disorders hitherto attributed to dopaminergic function in subcortical areas.

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Behavioural variants of rat neuroblastoma cells

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Study of the biological function of the cells of the nervous system has benefited from the isolation of cloned glial or neuronal tumour cell lines that can be induced to differentiate in culture¹⁻³. Many of their differentiated properties reflect specific biochemical changes at the cell surface, and we have observed changes in cytoskeletal organization and glycosaminoglycan-containing proteoglycan content in the tissue culture substratum adhesion sites of neuroblastoma cells during neurite extension⁴. To assist in defining structure function relationships of cell surface components of rodent neuroblastoma cells, I have used two cell-surface-active agents to select for phenotypically-stable variants of these cells which display some unique properties and which will be useful for further biochemical and physiological analyses.

The first selection, based on sensitivity to detachment of these cells from the tissue culture substratum by the Ca^{2+} -specific chelator EGTA, is shown in Fig. 1 for cloned rat neuroblastoma B104 cells⁵. Variants were isolated that resist EGTA-mediated detachment from the tissue culture dish on conventional treatment [shaking at 37°C for 30 min with 0.5 mM EGTA in phosphate-buffered saline (PBS)]. The selection was performed in two different ways. In the first approach (Fig. 1a), undifferentiated B104 cells were treated with EGTA, gently pipetted to detach loosely-adherent cells, and rinsed several times with PBS. Cells resisting this treatment (approximately 5×10^{-5} of the original cell number) were incubated for several hours in fresh medium and trypsin-subcultured into fresh dishes. After 2 weeks, several colonies developed at a final selection frequency of 1×10^{-5} ; these colonies were subjected to the same selection protocol two more times before cloning. Most of the colonies from this selection were resistant to EGTA at 0.5 mM and

displayed a highly elongated fibroblastic morphology. These cells are referred to as $\text{E}_\text{R}\text{A}$ variants; higher EGTA concentrations or longer trypsinization are required to subculture them. Several clones were tested and showed properties similar to those of the $\text{E}_\text{R}\text{A}$ 12 clone described here.

In an alternative procedure (Fig. 1b), cells in the original culture that resisted EGTA detachment were permitted to regrow into colonies in the original culture dish instead of being treated with trypsin and transferred to fresh dishes. The original dish was covered with cellular substrate-attached material (SAM), substratum adhesion sites which pinch off from the cell surface during EGTA-mediated detachment of the cells⁶; it has previously been shown that oncogenic virus-transformed fibroblasts respond differently to SAM-coated substrata compared to serum-coated substrata than do normal fibroblasts^{7,8}. SAM-coated substratum permits selection of a different type of variant, termed $\text{E}_\text{R}\text{B}$, at a frequency of $\sim 2 \times 10^{-5}$. Most clones displayed properties similar to the $\text{E}_\text{R}\text{B9}$ clone described here. Both variant types have been stable over 9 months of culture (25-30 passages).

A third selection method was based on the sensitivity of rat B104 cells to agglutination and killing by the lectin concanavalin A (Con A) using protocols similar to those found successful in generating Con A-resistant fibroblasts⁹. A confluent washed cell layer was treated with $20 \mu\text{g ml}^{-1}$ Con A for 16 h in serumless DMEM. The nonviable agglutinated cells were pipetted off the dish and the resistant cells (frequency of 5×10^{-6}) were permitted to regrow into colonies on the same

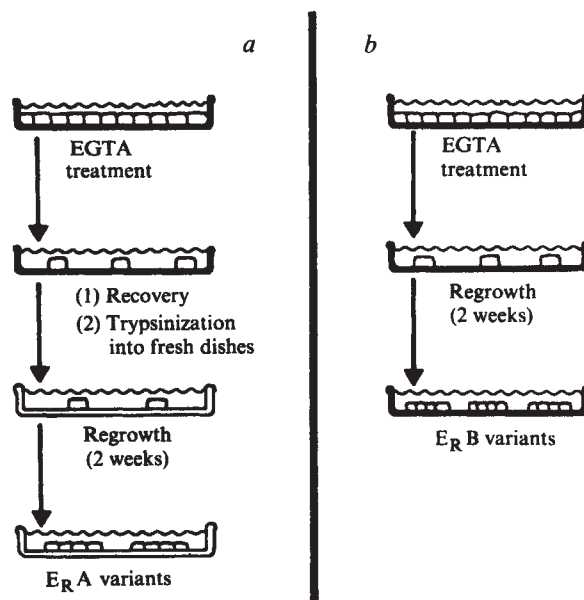


Fig. 1 Protocols for selection of EGTA detachment-resistant variants of rat neuroblastoma cells. Rat neuroblastoma cells (the B104 clone) were grown in 60 mm tissue culture dishes in DMEM (plus 7.5% fetal calf serum and penicillin and streptomycin) until they were just confluent ($\sim 4 \times 10^6$ cells). In both selection protocols (a and b), the cell layer was rinsed twice with PBS and treated with 0.5 mM EGTA in PBS at 37°C for 30 min by gentle shaking. Loosely-adherent cells were removed by gentle pipetting and the dish surface was rinsed twice with PBS. In protocol b, the dishes were refed with serum-containing medium to allow cell regrowth as a few sparse colonies on the SAM-coated substratum over a 2-week period. In protocol a, serum-containing medium was added back to the treated dishes for 4 h to permit recovery from EGTA-induced damage; the resistant cells were then trypsinized into fresh dishes to allow cell regrowth as a few sparse colonies over a 2-week period. Both of these protocols were repeated two more times before cloning of EGTA detachment-resistant cells.

Table 1 Growth and differentiation of variants

Property	Cell type and response			
	B104	C _R 4	E _R A12	E _R B9
(1) Density-dependent inhibition of growth*	-($>6\%$)§	-($>5\%$)§	-($>4\%$)§	+(1.2)
(2) Form neurites in serum-containing medium	-	-	-	+
(3) Form neurites in serumless medium†	+	+	-	+
(4) Form neurites on SAM-coated substrata‡	-	-	-	+

The designation + indicates that more than 25% of the cells responded; - indicates that less than 1% respond.

*As determined by multilayering of cells and continued high DNA synthesis in cultures after confluence of the culture had been achieved. The numbers in parentheses are saturating densities (cells per cm² $\times 10^{-5}$).

†At 50% confluence of the culture, the cell layer was rinsed twice with PBS and refed with DMEM lacking serum. Twenty-four hours later, the culture was microscopically evaluated for neurite formation.

‡SAM-coated substrata (on 60mm tissue culture dishes) were formed by growing rat C6 glioma or rat B104 neuroblastoma cells to confluence, detaching them by EGTA treatment, and rinsing the SAM layer with PBS before inoculating 5ml of serum-containing medium containing 5×10^5 cells. The cells were grown for 48h before evaluating whether they had formed neurites.

§Cells are multilayered at this density.

||These cells elongated and fused with neighbouring cells to form multinucleated syncytia.

dish. This selection was performed three more times with the lectin showing much less of a cytotoxic effect on each subsequent treatment. This variant is referred to as C_R4.

The growth properties and morphology of all four cell types were determined. The doubling time during exponential

growth was similar for 'wild-type' B104, E_RA 12, E_RB9, and C_R4 cells (18–20h). B104, E_RA 12 and C_R4 cells grew to a very high density with multilayering of cells (Table 1). E_RB9 cells differed in that they became density-inhibited when they formed a criss-cross array of neurites at higher density (Fig. 2d) without the cell body alignment characteristic of the parental B104 cells (Fig. 2a). They differed from the other cells in containing neurites at any cell density in serum-containing medium (Fig. 2c, d), except for an initial 12–24h recovery from subculturing damage. These cells are essentially constitutive for neurite formation and are less spread over the substratum than the E_RA 12 (Fig. 2e) or 'wild-type' B104 cells. C_R4 cells tend to be somewhat epithelioid in appearance (data not shown).

The ability of these cells to form neurites was then tested in various experimental conditions (Fig. 2 and Table 1). The B104 cells only formed neurites when they were starved of serum for 24h (Fig. 2b). They did not form neurites when grown in serum-containing medium on substrata coated with rat C6 glioma or neurite-containing B104 neuroblastoma SAMs (generated by prior growth of these cells to confluence and EGTA-mediated detachment). The E_RB9 cells contained neurites in all experimental conditions tested. The E_RA12 variant did not form neurites in any condition and underwent massive fusion within 24h in serumless medium to form highly elongated 'myotube-like' syncytia containing many nuclei. Also, at low density in serumless medium (Fig. 2f), the syncytia only contained 2–4 nuclei; with cells approaching confluence, the syncytia contained as many as 20 nuclei. These elongated cells frequently spanned the entire microscope field, and there was no evidence of growth cone formation in these cells. There was no appreciable syncytia formation when these cells were in serum-containing medium. C_R4 cells were similar to B104 cells in their ability to form neurites in serumless medium but not in serum-containing medium or during growth on SAM-coated substrata (Table 1). Thus, the selection protocol based on EGTA-mediated detachability and regrowth

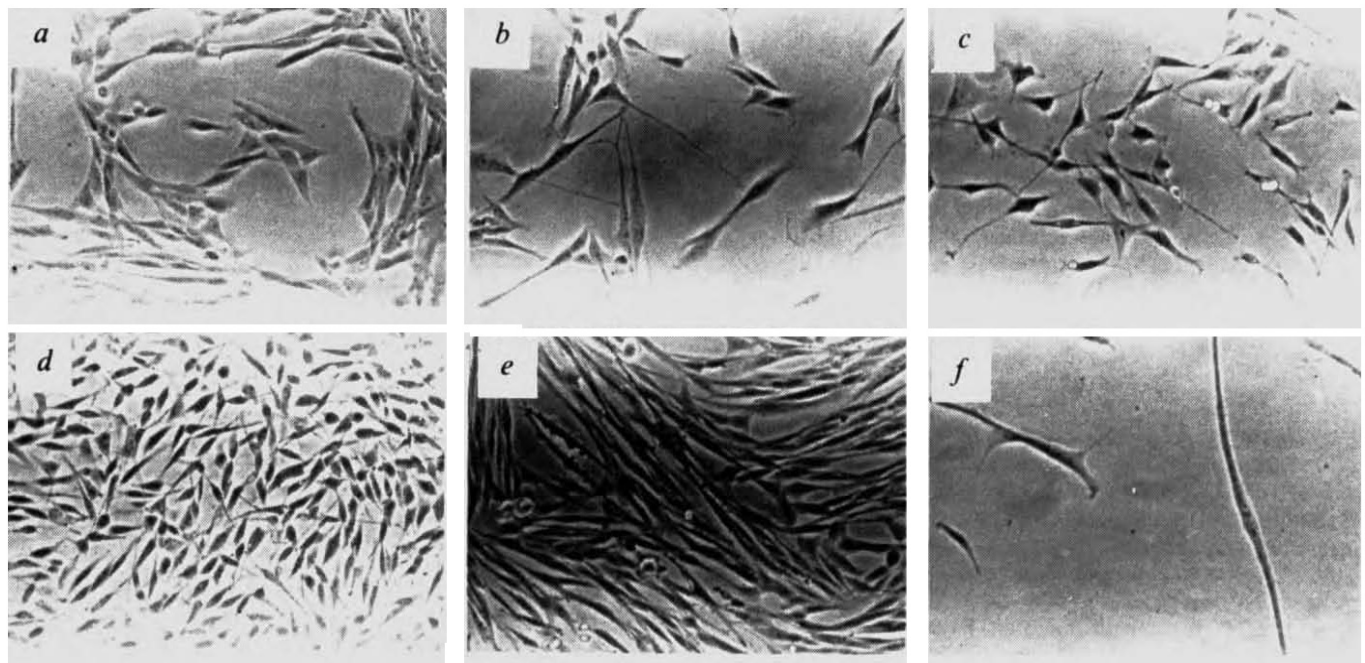


Fig. 2 Morphology and differentiation of EGTA detachment-resistant variant cells. Different cell types were plated into 60-mm tissue culture dishes at various densities and permitted to grow for at least 48h before photographing the cultures under phase contrast optics ($\times 90$). For serumless cultures, the cell layer was rinsed twice with PBS and refed with serumless DMEM for 24h before photography. The following are displayed: a, sparse B104 cells; b, neurite-containing B104 cells in serumless medium; c, sparse E_RB9 cells in serum-containing medium (note extensive neurite formation); d, density-inhibited E_RB9 cells in serum-containing medium (note the extensive array of criss-crossed neurites and the absence of alignment of cell bodies); e, E_RA12 cells as they approach confluence in serum-containing medium; and f, E_RA12 cells in serumless medium.

Table 2 Tumour formation in nude mice

Cell line	Tumour incidence		
	10 ⁴ cells	10 ⁶ cells	10 ⁷ cells
B104	4/4 (3)	4/4 (1.5)	4/4 (1.5)
C _R 4	4/4 (3.5)	4/4 (2)	4/4 (1.5)
E _R B9	0/4 (—)	4/4 (4)	4/4 (3)
E _R A12	0/4 (—)	0/4 (—)	0/4 (—)

Confluent cultures were rinsed twice with PBS and treated with 0.5 mM EGTA in PBS to detach cells. The cells were pelleted by centrifugation and washed once with serumless DMEM. The cells were suspended in serumless DMEM. 10⁴, 10⁶, or 10⁷ cells were inoculated subcutaneously into each athymic nude mouse in 0.1 ml aliquots. Animals were palpitated twice a week to detect tumour formation. Tumour incidence is given as the number of tumour-bearing animals (always resulting in animal death)/number of animals inoculated. The numbers in parentheses indicate the number of weeks required to grow a 1 cm-diameter tumour.

on two different substrata generated variants strikingly different in their ability to generate neurites; both types of variant were distinct from respective parent cells.

The variant cells were also tested for their ability to form tumours in athymic nude mice (Table 2). Three different inocula of EGTA-detached cells were used, and mice were examined for tumour formation over a 3-month period. At all three inocula sizes, 'wild-type' B104 and C_R4 cells formed tumours in all mice with comparable speed. In these experiments, tumour formation always led to death of the animal. Even though E_RB9 cells are density-inhibited in culture, they formed tumours in these animals, although more slowly than B104 cells. E_RA12 cells, on the other hand, did not form tumours in any of the animals even at the highest inoculum size (10⁷ cells); these cells are not density-inhibited in culture.

Thus the two EGTA selection protocols generated variants differing widely in tumorigenic potential and neurite differentiation. There is clearly no correlation between tumorigenesis and density sensitivity during *in vitro* growth. Regrowth of EGTA-treated cells on SAM-coated substrata selects for E_RB neurite-constitutive cells which apparently have lost the serum sensitivity for neurite formation as expressed in the parental cells; neurite extension and cell division are clearly not mutually exclusive events in these cells. The E_RA selection generates variants with an altered surface membrane that permits efficient cell fusion but not growth cone formation; the extensive elongation of these cells may also reflect some perturbation of cytoskeletal organization. Further study of these three variant classes should assist in establishing what cell surface properties are important in differentiation of these neural cells and in tumour formation in an appropriate host.

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Ammonia inhibits phagosome-lysosome fusion in macrophages

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When foreign bodies, including many microorganisms, are ingested by cultured macrophages, they become enclosed in phagosomes, with which lysosomes usually fuse and then discharge their enzymes and other contents into the resulting phagolysosomes. Such fusion is, however, diminished or absent after the phagocytosis of some pathogens, notably *Mycobacterium tuberculosis*¹ and *Toxoplasma gondii*². Assuming that the nonfusion is due to active inhibition by the intraphagosomal microbe, identification of an inhibitor should clarify the lysosomal control mechanism. It has been suggested that strongly acidic sulphatides present in virulent *tuberculosis*, which, like other substances with polyanionic structural features, can themselves block phagosome-lysosome fusion (P-LF)³⁻⁵, may contribute to the negative lysosome response to ingested tubercle bacilli⁴. We report here another possibility, based on inhibition of fusion of yeast-containing phagosomes by filtrates from cultures of tubercle bacilli on traditional-type defined media; we show that the ammonia content of such filtrates is sufficient to account for their effect. This inhibition of fusion seems to be an hitherto unrecognized intracellular consequence of added ammonia, in striking contrast to the enhancement produced by some lipophilic amines³.

Filtrates were obtained from surface-pellicle cultures of either *M. tuberculosis* virulent human strain H37Rv after 2-4 months on Ratledge-Hall 'normal' liquid medium⁶, containing asparagine as sole nitrogen source, or *M. tuberculosis bovis* strain AN5 after 10 weeks on a modified Dorset-Henly liquid medium of similar composition. Lysosomes of mouse peritoneal macrophages in monolayer culture were prelabelled with acridine orange (AO) for darkfield vital fluorescence microscopy (VFM), or with ferritin for electron microscopy (EM)³. The cells were allowed to ingest fresh or 150-krad γ -ray-irradiated *Saccharomyces cerevisiae*, an organism which induces P-LF. Fusion was assessed by transfer of label into the yeast-containing phagosomes. With this test system (see ref. 3 for details), substantial inhibition of P-LF was obtained when the monolayers were exposed to either type of culture filtrate (diluted 1:5 or 1:10 in balanced salt solution (BSS)⁷) at 37 °C for 60 min immediately before the yeasts were offered for ingestion and then continuously until examination 45-60 min later (exposure to culture filtrate not being interrupted at any stage).

Experiments involving distillation, trapping in H₂SO₄ and gas chromatography suggested activity by a volatile base. Therefore, ammonia, a known constituent of *M. tuberculosis* cultures⁸, was estimated in the culture filtrates, using glutamic transaminase and NADH (Sigma kit 170-UV), and was found to be as high as 20 mM. Replacement of culture filtrate by ammonia was tried and found to be effective in inhibiting P-LF, both by overall assessment using VFM and by quantitative assessment (based on screening surveys¹) using EM (Table 1a, b). (Quantitative differences in estimating fusion between these two methods, and between fresh and irradiated yeasts, have been discussed elsewhere³.) Corresponding to the formation of phagolysosomes by P-LF, degranulation (loss of lysosomes) takes place. Assessed by labelling the surviving lysosomes with AO, degranulation was much reduced by ammonia treatment of macrophages (Table 1c).

Ammonia rapidly enters lysosomes of macrophages and leaves rapidly after removal from the medium⁹. In the present

Table 1 Effect of ammonia on phagosome-lysosome fusion and degranulation in macrophages after ingestion of yeasts

Protocol	Optical system	Observation	Fusion or degranulation (%)	
			Untreated	Ammonia-treated
a	Fluorescence microscopy (AO label)	Phagosome-lysosome fusion	70	10
b	Electron microscopy (ferritin label)	Phagosome-lysosome fusion	92*(350)†	55*(390)†
c	Fluorescence microscopy (AO label)	Degranulation	90	25

a, Macrophage monolayers, after prelabelling with AO, were treated for 45 min with NH_4Cl (10 mM) in BSS at 37°C and then exposed for a further 50 min to fresh yeasts at 10^6 cells per ml, the NH_4Cl still being present. (The results given are representative of 20 experiments, in 4 of which inhibition was evident using 1–2 mM NH_4Cl .) b, Macrophage monolayers, after prelabelling with ferritin, were treated for 45 min with NH_4Cl (5 mM) in BSS, then exposed for 30 min to irradiated yeasts (10^7 cells per ml). After washing to remove excess yeasts, incubation was continued for 45 min and the macrophages were then fixed. NH_4Cl (5 mM) was present throughout (including the yeast pulse and the washing). c, Similar to a but the yeasts, at 5×10^7 cells per ml, were applied for 30 min only, after which incubation was continued for 150 min, NH_4Cl (10 mM) being present throughout all stages; AO labelling was at the end of this period, immediately before examination. Figures denote percentage loss of AO-labelled lysosomes (results are representative of six experiments).

*Difference between results is significant ($P < 0.01$).

†Number of individual phagosome profiles counted.

work the lysosomes were seen to swell to a degree dependent on dose (evident by VFM and EM). At concentrations up to 10 mM for 2–3 h at 37°C, the cells remained apparently healthy by light microscopy, with normal uptake of yeasts; by EM, autophagic vacuoles were few and mitochondria seemed normal in appearance. Moreover, when ammonia-treated macrophages that showed inhibition of P-LF were washed well with BSS and incubated for several hours, the lysosomal swelling declined and normal fusion pattern was restored.

Ethylamine and methylamine hydrochlorides were tested in the yeast system and showed some inhibitory activity. In view of this, and the behaviour of other amines tested by us previously, an interesting series with respect to effect on P-LF emerges. Lipophilic secondary or tertiary amines (for example, di- and tri-*n*-butylamine, dihexylamine, dibenzylamine, chloroquine and tetracaine) accelerated P-LF; lower tertiary and secondary amines (such as diethylamine and triethylamine) enhanced slightly; most primary amines tested (for example, *n*-butylamine and ethanolamine) also enhanced slightly³. Now we have the opposite effect, that is, some inhibition by the lower primary amines, ethylamine and methylamine, and much stronger inhibition by ammonia. The negative or weakly positive results of testing individual amines other than ammonia for inhibitory activity are in accord with further experiments with culture filtrate which indicate that the latter's ammonia content is sufficient to account for most, if not all, of its fusion-inhibitory activity in the yeast system. These experiments comprised comparison of activities of NH_4Cl and culture filtrate at equivalent ammonia concentrations, and repetition of the trapping experiment mentioned above, but after preliminary removal of the ammonia by treatment with glutamic dehydrogenase, NADH and α -ketoglutarate.

Ammonia raises intralysosomal pH (ref. 9) and inhibits degradation of lysosomal proteins^{10–12}; so also do some other bases, including methylamine, tributylamine and chloroquine^{9–11,13,14}. The present observation that ammonia has the opposite effect on P-LF to that of chloroquine and tributylamine makes it unlikely that its inhibitory activity

operates through interference with pH and protein metabolism.

Thus, judged by fluorescence and electron microscopy, and by lysosomal degranulation, ammonia can inhibit P-LF in macrophages when *S. cerevisiae* is the target. Moreover, *M. tuberculosis* produces sufficient ammonia when grown *in vitro* to account for most or all of the fusion-inhibitory effect of its culture fluid in this yeast system. The possibility that *M. tuberculosis* achieves its fusion-inhibitory effect by the production of ammonia or relevant amines within phagosomes of macrophages is now being studied.

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Does the variability of the cell cycle result from one or many chance events?

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Smith and Martin^{1,2} proposed the transition probability (TP) model to explain the significance of the variability of the cell cycle length of individual cells within a clone or culture. This postulates that the cell cycle length consists of a fixed period of time, which is the same for all members of the population, plus a variable period which is distributed according to a negative exponential function among the individual cell cycles. This variable part is contained entirely within G1, may be controlled hormonally by the organism, and is assumed to result from a single first order random process that terminates the variable part of the cell cycle with constant probability. As originally proposed, the model failed to account for timing of the fastest third of the population³. Minor and Smith⁴ assumed that there is biological variability in the previously presumed constant part of the cycle and that this variability accounts for the failure to see an abrupt discontinuity. They proposed that most of this kind of variation can be cancelled out by comparing sister cells. Thus, they proposed a second plot to test their model, the β -plot. This is a two-cycle semi-logarithmic plot of the percentage of sister-sister cells having a smaller difference in age at division than the time value corresponding to the abscissa of the graph. Their model predicts a straight line starting at 100% at time zero. Any model would require this value at time zero, but theirs requires that the plot be straight and parallel to the terminal slope of the α -plot. Shields and Smith⁵ and Shields⁶ have convincingly shown that for tissue culture cells of both mouse cell lines and *Staphylococcus albus*, the β -plots are linear for 97–98% of the cell populations and parallel to the α -plots. This has been taken as strong support for the TP model. Recently, in

developing computer simulation of a growth controlled (GC) model⁷, I found that certain properties, such as the linearity of β -plots, are very sensitive to the presence of subpopulations of cells with longer doubling times. My major purpose here is to point out three statistical aspects of the cell cycle that are strongly influenced by growth rate heterogeneity for any model of the cell cycle.

The GC model as used here assumes that the sizes at successive divisions are uncorrelated and that the cell division produces daughter cells of equal size. I have assumed both a gaussian normal distribution and a negative exponential distribution and that growth of individual cells may follow exponential, linear or bi-linear growth laws. The negative exponential distribution was chosen because it is non-normal, moderately skewed in the positive direction and is the form assumed by the TP model. In addition, such a distribution would apply if the actual separation of the completed daughter cells proceeds by a process whereby the splitting into two separate cells has a constant probability with time⁸ after the cells had physiologically divided.

Figure 1 shows computer-generated α - and β -plots for various combinations of distributions and growth laws. Calculation has been made for the case in which the coefficient of variation of the sizes of cells in the act of division (cv_d) is 0.1. It can be seen that the negative exponential-linear growth model gives linear β -plots; it is, in fact, equivalent to the TP model. However, the α -plot for this case has a quite different terminal slope. When exponential growth is assumed, both choices for the distributions of sizes at division give parallelism between the β -plot and the terminal part of the α -plot, but both are curved downwards. Table 1 gives the statistical parameters of these distributions and other statistics for these submodels.

For a single case, that of the prokaryote, *Escherichia coli*, there are reasons independent of the statistical data for choosing the GC model corresponding to the first line of Table 1. The distribution of sizes of cells showing constriction in electron micrographs is approximately gaussian and not exponential^{9,10}. The coefficient of variation of sizes of cells engaged in cell division is small ($cv_d=8-14\%$)¹¹⁻¹³; the rate of macromolecular synthesis is exponential¹⁴. Evidence for equality of cell division in producing equal-sized daughters is presented elsewhere^{10,13}, as are other types of evidence in favour of the model^{12,13,15}.

Some, but not all, of the statistical data for *E. coli* concerning cell cycle lengths agree with this GC submodel. The coefficient of variation of the distribution of ages at division ($cv_e=20-25\%$) is very nearly twice the coefficient of variation of the sizes of cells at division ($cv_d=8-14\%$)¹³. The model also predicts values of ρ_{ss} , the correlation coefficient of

sister:sister ages at division of about 0.5 (see first line of Table 1). Values this high or higher have been observed in all studies except where cell division is very asymmetrical.

Certain experimental findings with *E. coli* and other prokaryotes are not predicted but are generally observed. They are: (1) that the measured correlation of ages at division of mother and daughters, ρ_{md} , is usually observed to be more positive as values near -0.5 predicted by the model (Table 1); (2) that the measured skewness of the distribution of ages at division, γ_1 , is generally moderately positive instead of being nearly zero as predicted by the theory (Table 1); (3) that the β -plot is linear instead of exhibiting a downward curvature (see Fig. 1). Other choices of growth laws or distributions of division sizes do not give agreement (see Table 1 and Fig. 1). However, these three discrepancies can be fully resolved in a way which leaves the basic model intact by assuming that a small fraction of the cells in the population grow more slowly than do the majority. From Table 1 it can be seen that admixture with 2-3% more slowly growing cells decreases ρ_{md} and increases γ_1 towards values typically observed in the literature. Figure 2 shows α - and β -plots for the gaussian-exponential GC model with $cv_d=0.1$ repeated from Fig. 1 together with the plots for hypothetical populations in which

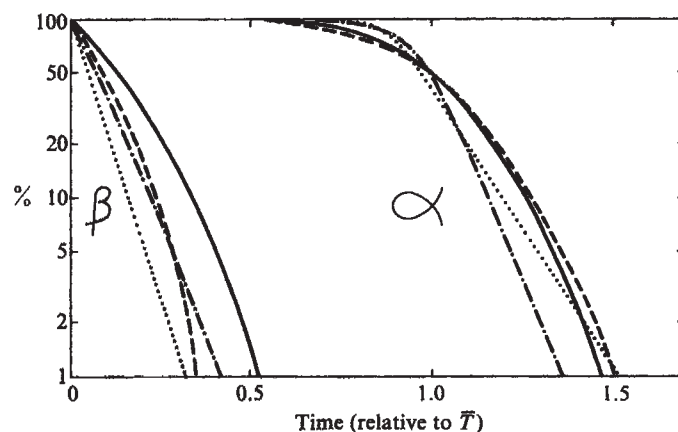


Fig. 1 α - and β -plots for four submodels of the growth controlled model of the cell division cycle. It was assumed that the coefficient of variation of the sizes of cells at division, cv_d , is 0.1 and that cell division partitions cell substances precisely. The submodels differ in the shape of the distribution of sizes at cell division and the mode of growth:

Distribution	Growth law	
Gaussian	Exponential	—
Gaussian	Linear	- - -
Negative exponential	Exponential	- . - .
Negative exponential	Linear	

Table 1 Statistical properties of growth controlled models of the cell cycle

Division distribution	Growth law	Coefficient of variation of the age distribution cv_e	Skewness γ_1	Kurtosis† β_2	Sister : sister correlation ρ_{ss}	Mother : daughter correlation ρ_{md}
Gaussian	Exponential	0.204	-0.001	2.989	0.501	-0.501
Admixed with: 2% delayed 0.57 Gen.		0.217	+0.271	3.758	0.775	-0.226
3% delayed 0.52 Gen.		0.219	+0.305	3.697	0.877	-0.125
Gaussian	Linear	0.221	0	2.886	0.200	-0.400
Gaussian	Bi-linear*					
C = 1.3		0.215	0	2.916	0.501	-0.501
C = 1.6		0.175	0	2.916	0.501	-0.501
Negative exponential	Exponential	0.136	0	4.651	0.502	-0.501
Negative exponential	Linear	0.157	+1.196	6.418	0.200	-0.399

All models are calculated for the assumption that the size of cells at division has a coefficient of variation (cv_d) of 0.1.

*C equals the size of the cell at which the rate of growth doubles relative to the mean birth size.

†For a normal distribution $\beta_2=3$.

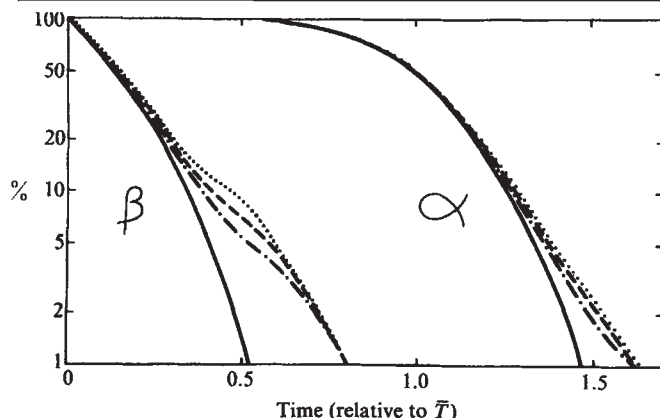


Fig. 2 The effect of straightening the β -plot by the admixture of a few per cent of more slowly growing cells. Calculations are for the gaussian distribution of sizes at division, exponential growth and $cv_d=0.1$. —, No admixture; ---, 2% delayed 0.57 of $\bar{T}$; - · - ·, 3% delayed 0.52 of $\bar{T}$; · · · ·, 4% delayed 0.49 of $\bar{T}$.

2, 3 or 4% are presumed to be delayed in division for about 0.5 of a mean generation compared with the majority population. In each percentage of admixture, I have fitted for the delay that gives the most linear β -plot.

The following conclusions follow from these considerations. (1) A small subfraction of slowly growing cells can greatly affect some but not others of the statistical properties of the life-lengths of cells in a growing culture. The coefficient of variation of the ages at division, and its relationship to the variation in size at division are not as responsive to the presence of a small proportion of more slowly growing cells. (2) The mother-daughter correlation, ρ_{md} , the skewness and kurtosis of the age distribution, γ_1 and β_2 , and the curvature of the β -plots are very sensitive to admixture and therefore give little information about the events timing the cell cycle of the bulk of the cells of the population. (3) If there were a very few per cent of a population growing more slowly than the bulk of the cells, the transition probability model would fit the available data for a variety of cell lines very poorly. (4) In one case, that is, for *E. coli*, the gaussian-exponential submodel of the growth controlled model is known to apply because of the results of measurements not involving cell cycle length observations. These measurements involve electron microscopy, molecular biological measurements and autoradiography. In addition, the statistical properties not sensitive to admixture are consistent with the model. Consequently, for this case we believe that admixture of a small amount of a more slowly growing population accounts for the properties mentioned in (2). (5) Because it is virtually impossible to eliminate the possibility of a small, slowly growing subfraction experimentally, the measures mentioned in (2) are less useful and should not be used as a point of argument. (6) It is argued that instead, measurements of the distribution of cell properties at division and at other critical times in the cell cycle, and the growth kinetics of individual cells throughout the cell cycle be used as the basis for cell division models that apply to the majority class of cell populations as other cell types are studied. (7) Although the TP model may function for some cell lines, a single stochastic even cannot be adduced as timing the cell division cycle in any cell line based on the available types of data.

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The insulin gene is located on chromosome 11 in humans

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The polypeptide hormone insulin is required for normal glucose homeostasis. Insulin deprivation results in diabetes, a disease affecting up to 5% of the human population^{1,2}. In some animals there are two insulin genes³; however, in most others, including humans, a single gene is present. The conclusion that there is a single insulin gene in humans is based on the finding of a single insulin species and on genetic studies involving mutant insulin alleles^{4,5}. As the pattern of inheritance of insulin defects is not sex linked, the insulin gene must be located on an autosomal chromosome⁵. Recently, genomic DNA segments containing the human and rat insulin genes have been cloned and the DNA has been sequenced⁶⁻⁸. The fact that mouse and rat insulins are identical³ suggests that the insulin gene sequences and organization are similar. A knowledge of the restriction endonuclease map for the human and mouse insulin genes enables human or mouse insulin DNA sequences to be distinguished in somatic cell hybrids between the two species. In the present study, somatic cell hybrid gene mapping methodologies⁹ have been used to determine the chromosome localization¹⁰ of the human insulin gene. Human-mouse cell hybrids with different numbers and combinations of human chromosomes were examined for the presence of specific human chromosomes and the human insulin gene using cloned human and rat cDNA probes. A 14-kilobase DNA fragment containing the human insulin gene was present in some cell hybrids and was easily distinguished from the DNA fragments containing the mouse insulin gene found in all cell hybrids. Co-existence of the 14-kilobase fragment and chromosome 11 indicate that the insulin gene resolves on chromosome 11 in humans.

Human and mouse parental cells were fused with β -propiolactone-inactivated Sendai virus or polyethylene glycol in monolayer culture and hybrid cells were cloned and propagated in hypoxanthine, aminopterin, thymidine (HAT) selection medium (see ref. 9). The cell hybrids were isolated and characterized by isozyme and karyotype analysis (Table 1)⁹. Genomic DNA was prepared from 15 cell hybrids, each containing a full complement of mouse but a reduced number of human chromosomes. This DNA was digested to completion with *EcoRI* restriction endonuclease. The fragments were separated by agarose gel electrophoresis, and then transferred to filters by the method of Southern¹¹. Cloned cDNA fragments containing the coding sequences for rat⁷ and human¹² preproinsulin mRNA were labelled by nick translation¹³ and used as probes for insulin gene sequences in the DNA from human/mouse hybrid cells. Filters were hybridized and washed as described in Fig. 1 legend. Although the human probe cross-hybridizes with mouse DNA sequences, preliminary experiments showed that the best resolution of both human and mouse insulin sequences in cell hybrids was obtained by using a mixed ³²P-labelled probe to both rat and

Table 1 The distribution of human insulin, LDH A and human chromosomes in human-mouse cell hybrids

Hybrid	Insulin†	LDHA‡	Chromosomes*																						9/X	7/9	X/15	15/X	17/3
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22					
WIL-2	—	—	—	—	—	—	—	—	+	—	+	—	+	—	+	—	+	—	—	+	—	+	—	+	—	—	—		
WIL-8Y	+	+	—	—	—	—	+	+	—	—	+	+	—	—	+	—	—	+	+	—	+	+	—	+	—	—	—		
TSL-2	—	—	—	+	—	—	+	+	—	—	+	—	+	—	—	—	—	+	+	—	+	+	—	+	—	—	—		
TSL-5	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	—	—	+	+	—	+	+	—	+	—	—	+		
TSL-6	—	—	—	—	—	+	—	—	—	—	+	—	—	—	—	—	—	+	+	—	—	—	—	—	—	—	+		
TSL-8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—	—	—	—	—	+		
RAS-8	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—		
RAS-9DT	+	+	+	+	+	—	+	+	+	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+	—	—	—		
SIR-8	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+		
XTR-3BSAgA	—	—	—	—	+	+	+	+	+	+	+	—	—	+	—	—	+	+	+	—	+	—	—	—	—	—	—		
DUA-5	+	+	—	—	+	—	+	—	—	—	—	+	—	—	+	+	—	+	+	—	—	+	—	—	—	—	—		
DUM-13	+	+	+	+	+	—	+	+	+	—	+	+	—	+	—	+	+	+	+	+	+	+	+	+	—	—	—		
ALR-2	+	+	+	—	+	+	+	+	+	—	+	+	+	+	+	+	+	+	—	+	+	+	—	—	—	—	—		
JSR-6C	—	—	—	+	+	—	+	—	—	—	+	—	—	—	+	+	+	+	—	+	—	—	—	—	—	—	+		
JSR-24D	+	+	+	—	+	—	+	+	—	+	—	+	+	—	+	+	+	+	+	+	+	+	—	+	—	—	+		

Chromosomally characterized human cell lines were used for parental cells. WIL hybrids (human WI-38 $\times$ mouse LM/TK⁻), RAS hybrids (mouse RAG $\times$ human SH 421) and SIR hybrids (human GM 469 $\times$ mouse RAG) are derived from chromosomally normal human parental cell lines²²⁻²⁴. The remaining hybrid sets were derived using chromosomally characterized human translocation lines for regionally localizing human genes. TSL (GM 2808 $\times$ LM/TK⁻) hybrids involve the [46,XX,t(3;17)(p21;p13)] translocation and segregate the 17qter $\rightarrow$ 17p13::3p21 $\rightarrow$ 3pter translocation²⁵. XTR (GM 2899 $\times$ RAG) hybrids involve the [46,X,t(X;3)(q26;q13)] translocation²⁶. DUA (DUV $\times$ A9) and DUM (DUV $\times$ RAG) hybrids involve the [46,X,t(X;15)(p11;q11)] translocation and segregates the Xqter $\rightarrow$ Xp11::15q11 $\rightarrow$ 15qter and 15pter $\rightarrow$ 15q11::Xp11 $\rightarrow$ Xpter chromosomes²⁷. The ALR (AnLY $\times$ RAG) hybrids involve the [46,X,t(X;9)(q12;p24)] translocation and segregates the Xqter $\rightarrow$ Xq12::9p24 $\rightarrow$ 9qter chromosome²⁸. The JSR (JoSt $\times$ RAG) hybrids involve the [46,XX,t(7;9)(q22;p24)] translocation and segregates the 7pter $\rightarrow$ 7q22::9p24 $\rightarrow$ 9pter chromosome²⁹.

*In each hybrid, chromosomes have been determined by Giemsa-trypsin staining²⁸ and follow the Paris system of human nomenclature²¹. In addition, enzyme markers whose genes have been assigned to each human chromosome, except the Y, have been tested on each hybrid and confirm the chromosome analysis (data not shown)^{9, 23, 32}.

†The insulin gene was measured as the 14-kilobase fragment.

†The LDH A gene has been assigned to chromosome 11 by several laboratories (see ref. 30) and co-segregates with the insulin gene.

human insulin genes and washing the filters at high stringency.

In the control mouse cell line, three DNA fragments of 9, 1.8 and 1.4 kilobases hybridized with ^{32}P -labelled rat-human insulin probe (Fig. 1, channel 1). These results are similar to those obtained with rat DNA^{7,8}. As both mouse and rat have two non-allelic insulin genes³, and as one of the rat insulin genes contains an *EcoRI* site^{7,8}, the simplest interpretation of the data is that one of the mouse insulin genes also contains an *EcoRI* site. Thus, three DNA fragments containing insulin-specific sequences would be detected.

In the control human cell line (Fig. 1, channel 2), and in all human parental lines used in this study, a single *EcoRI* fragment of 14 kilobases hybridized with the rat-human ³²P-insulin probe. This agrees with the findings of Bell *et al.*⁶, who showed that the human insulin gene is contained in a 14-kilobase *EcoRI* fragment. The presence of a single-size class of *EcoRI* fragments of human DNA containing insulin gene sequences also provides additional support for the existence of a single insulin gene in humans.

DNA from all 15 cell hybrids contained the mouse *EcoRI* fragments, whereas only some had the 14-kilobase human fragment (see Fig. 1 for representative data). The absence of the 14-kilobase *EcoRI* human fragment indicated the absence of the human chromosome on which the insulin gene is located. As the mouse sequences served as an internal hybridization control, the absence of the human 14-kilobase fragment was not due to other factors such as poor DNA transfer from the agarose gel or loss of DNA from the nitrocellulose filter. The segregation of the human insulin gene, human chromosomes and the chromosome 11 enzyme marker, lactate dehydrogenase A(LDH A) in the 15 human-mouse cell hybrids is shown in Table 1. All 22 autosomes and the X and Y chromosomes were scored both by karyotype and isozyme analysis (Table 1, see also ref. 9). For example, three hybrids (WIL-8Y, TSL-5, DUA-5) contained the human insulin gene but did not retain chromosome 1; it is thus obvious that the insulin gene is not on chromosome 1. The 14-kilobase human insulin fragment was invariably found when human chromosome 11 and LDH A were present in the cell hybrid (Table 1). None of the other chromosomes or enzyme markers co-existed with the 14-kilobase fragment (Table 1). The correlation of human chromosome 11 and the human insulin gene and the lack of correlation with any other human

chromosome, conclusively demonstrate the localization of this gene on chromosome 11.

Restriction analysis of somatic cell hybrid DNA is a very useful addition to genome mapping technology. Any gene for which a molecular probe is available can be mapped. Several laboratories have mapped the β -globin genes to the short arm of chromosome 11 (refs 14–17). Our procedure for mapping the human insulin gene includes the use of 10% dextran sulphate in the hybridization mixture, which increases the rate and extent of hybridization with the probe¹⁸. With 10 μ g of cell hybrid DNA and 3×10^4 c.p.m. of probe per cm² filter, it is possible to score the insulin-containing DNA fragment in less than 24 h exposure to X-ray film. These conditions permit the use of small numbers of cells ($3\text{--}6 \times 10^6$) which can be obtained either from cell culture or from cells previously frozen for recovery in 10% glycerol, 20% fetal calf serum and tissue culture medium. These frozen cells, previously typed by chromosome and enzyme analyses, can be thawed, washed and used as a source of DNA without growing additional cells and rechecking the chromosome and enzyme complements. Finally, the use of probes and hybridization conditions that detect both mouse and human genes permit the use of mouse genes as an internal standard, thus eliminating external artefacts.

The causes of idiopathic diabetes mellitus are largely unknown. Some forms of the disease can be the consequence of the destruction of insulin-producing β cells of the pancreas after viral infection or by chemicals^{1,2}. One example of diabetes, resulting from the production of a mutant insulin with altered biological properties, has also been reported¹⁹. In juvenile-onset or insulin-dependent diabetes a gene on chromosome 6 near the human lymphocyte antigen D locus (HLA-D) may influence the susceptibility of this form of the disease². The present work demonstrates this locus is not in the vicinity of the insulin gene. There is also a hereditary disposition to the most common form of the disease, maturity-onset or insulin-independent diabetes^{1,2}. If maturity-onset diabetes is a consequence of an altered insulin gene, the chromosome localization predicts that there should be a correlation among related diabetic individuals between diabetes and alleles of closely linked genes present on chromosome 11. Several genes have already been localized to specific regions of chromosome 11. The insulin gene may be precisely localized on chromosome 11 by examining hybrids

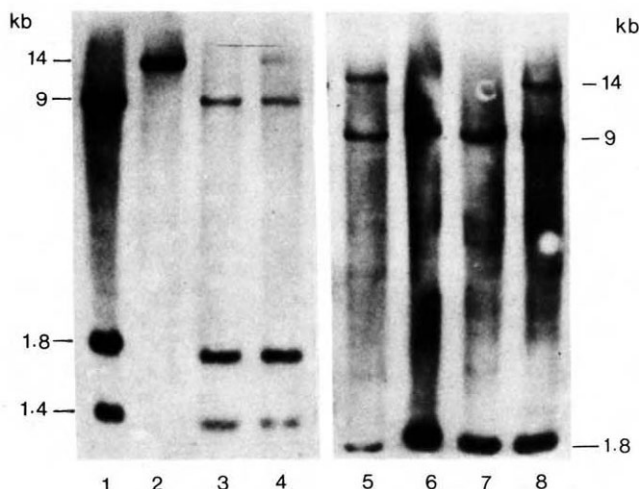


Fig. 1 Analysis of human and mouse insulin related DNA fragments present in restriction endonuclease digests of mouse, human and human-mouse cell hybrid DNA. DNA was prepared from a human T-cell lymphoblastoid cell line, RAG mouse cells and the 15 cell hybrids listed in Table 1. Approximately 4×10^6 cells of each hybrid clone were obtained from cells previously stored frozen in 10% glycerol, 20% fetal calf serum and tissue culture medium and were thawed, washed three times with Hank's balanced salt solution and DNA prepared as previously described²⁰. Hybrid cells collected from monolayer culture were also used and prepared in the same way. 20–40 μ g of high molecular weight DNA was obtained from 4×10^6 cells. The DNA was digested to completion with endonuclease *EcoRI*. Digested DNA (10–20 μ g) was separated by electrophoresis through a 1% agarose slab gel and transferred by the method of Southern¹⁵ to a nitrocellulose filter. pCRI-354 and pCHI-1 were labelled with 32 P *in vitro* by nick translation¹⁷ with [α - 32 P]dCTP and [α - 32 P]dATP (NEN) to specific activities of $1\text{--}3 \times 10^8$ c.p.m. per μ g. Before hybridization, nitrocellulose filters were treated for 2 days at 42°C in 10 ml of a mixture of 50% deionized formamide, 5× SSC (0.75 M NaCl, 75 mM trisodium citrate), 5× Denhardt's reagent²¹, 50 mM sodium phosphate buffer (pH 6.5) and 500 μ g ml⁻¹ sonicated denatured salmon sperm DNA (Sigma). Filters were then hybridized overnight at 42°C in a 10 ml solution consisting of 50% deionized formamide, 5× SSC, 1× Denhardt's reagent, 20 mM sodium phosphate buffer (pH 6.5), 200 μ g ml⁻¹ sonicated denatured salmon sperm DNA, 10% sodium dextran sulphate 500 (Pharmacia)¹⁸ and 2.5 ng ml⁻¹ each of heat-denatured 32 P-labelled rat and human insulin cDNAs. Filters were then washed three times at room temperature with 2× SSC, 0.1% SDS for 10 min each followed by three additional washes with 0.1× SSC, 0.1% SDS at 50°C for 15 min each. Labelled DNA bands were detected by exposing the filters to X-ray film for 1–5 days in the presence of an intensifying screen (Dupont). The insulin DNA patterns are shown for: (1), mouse RAG cells; 2, human T-cell lymphoblasts; 3, hybrid TSL-2; 4, hybrid SIR-8; 5, hybrid DUA-5; 6, hybrid JSR-6C; 7, hybrid TSL-8; 8, hybrid TSL-5. Fragments of *HindIII*-digested λ DNA were used as molecular weight markers. Channels 5–8 were run on a different slab gel and the 1.4-kilobase (kb) mouse insulin DNA fragment migrated off the gel. On long exposures, the autoradiographs of several hybrids (for example, channels 6, 7) showed high backgrounds. On shorter exposures, using the 9.0-kilobase mouse insulin fragment as a reference point, the 1.4-kilobase region could be accurately scored.

bearing translocations of this chromosome. Extensive information on the genetic loci of chromosome 11 may eventually allow the use of chromosome 11 markers to predict the probability of diabetes in individuals. Eventually, all or parts of chromosome 11 may be used in genetic engineering experiments for correcting diabetes in affected individuals.

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Electrophoresis of proteins in intercellular bridges

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Cytoplasmic polarity giving rise at mitosis to daughter cells with distinct but complementary morphogenetic functions has been proposed by Jaffe and co-workers to have transcellular ion currents as one of its essential physiological steps¹. The clearest evidence is from fucoid eggs in which such currents have been shown to parallel the prospective axis of germination^{2,3}. The currents are caused by stabilized accumulations of cation pumps on one side of the cell, and of permeability channels on the opposite side⁴; they are strong enough to suggest that the accompanying gradient in electrical potential could have an electrophoretic effect on the distribution of cytoplasmic constituents, including those that serve as morphogenetic determinants. Although ion currents correlated with axes of morphogenesis have thus been clearly established, their effects on intracellular localizations and morphogenetic activity have remained speculative⁵. We report here evidence that an endogenously generated gradient in electrical potential in the oocyte–nurse cell syncytium of *Hyalophora cecropia* can, in fact, influence the distribution of soluble proteins in a cytoplasmic continuum.

The system consists of seven nurse cells linked to each other and to a single oocyte by seven cytoplasmic bridges, which are clustered in a complex at the centre of the group (Fig. 1). The mitotic divisions giving rise to the eight cells entail incomplete cytokinesis, and the cells subsequently remain in cytoplasmic continuity throughout their 9-month life span⁶. By the time vitellogenesis begins in the oocyte, the bridges are 30- μ m wide channels containing no membrane barriers to intercellular diffusion, and little evidence of orientated cytoskeletal elements in their core of cytoplasm⁷. Despite this structural simplicity, nurse cell products migrate into the oocyte in an apparently polarized fashion^{8,9}. The bridges provided an opportunity to test for intracytoplasmic electrophoresis because, as we showed previously⁷, the equilibrium potential of the nurse cells is several millivolts more negative than that of the oocyte. Furthermore, a negatively charged, fluorescein-labelled, rabbit serum globulin (FSG), when micro-injected into the oocyte, was unable to cross

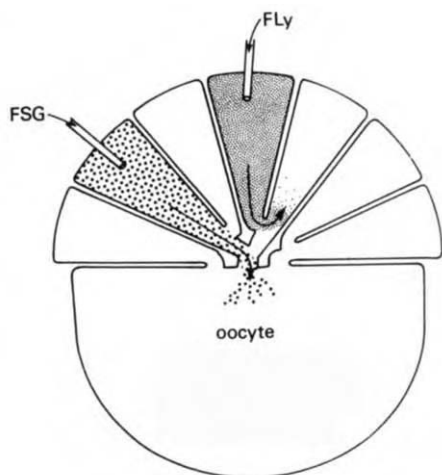


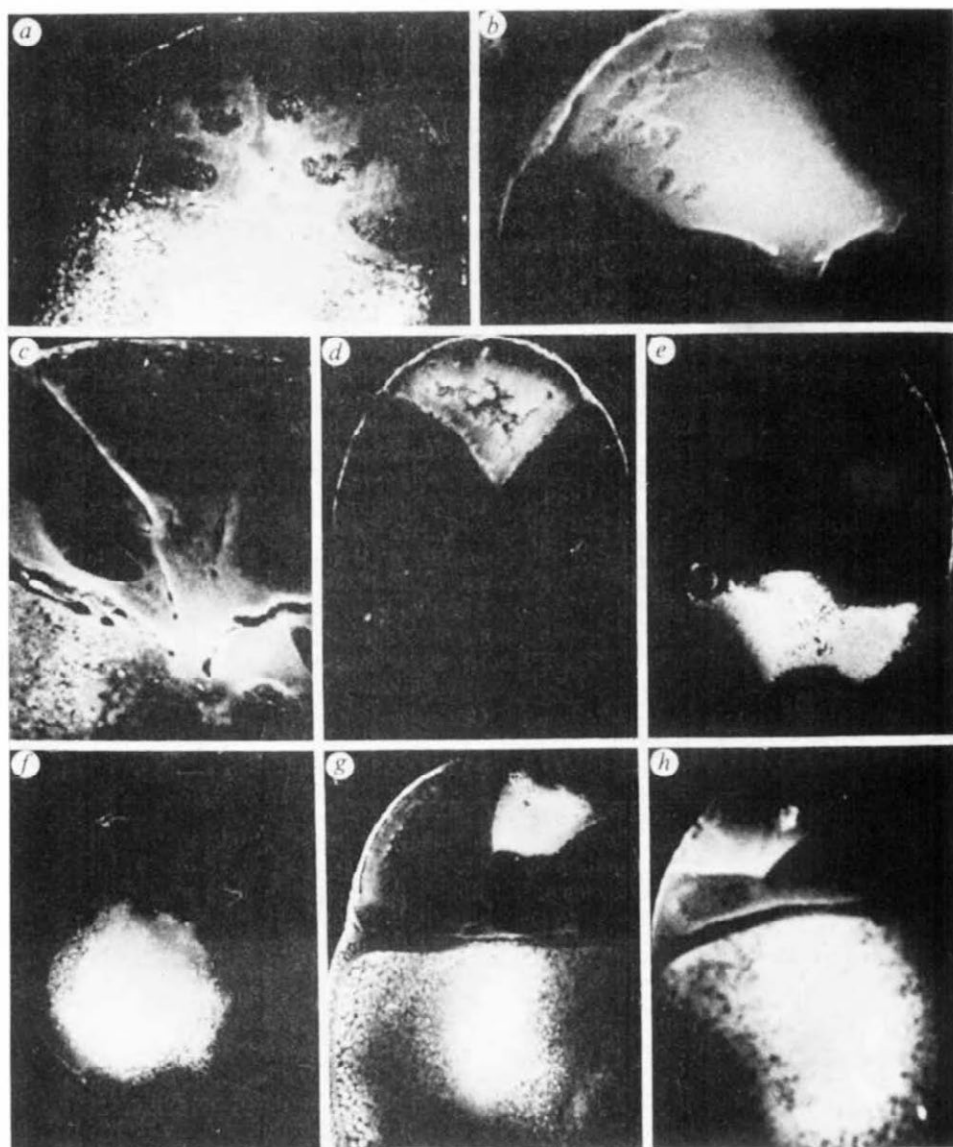
Fig. 1 Diagram of the oocyte and seven nurse cells and the distribution of the seven intercellular bridges that connect them. Also shown are examples of the behaviour of fluorescent serum globulin (FSG) and lysozyme (FLy) after injection into nurse cells.

the bridges into the nurse cells, although it readily moved in the opposite direction when injected into a nurse cell (Fig. 1).

These results showed definitively that the bridges are polarized both electrically and in their ability to transmit protein. Although they were thus consistent with the potential gradient being the polarizing force, satisfactory proof of this proposal needs evidence that basic proteins move through the bridges in the opposite direction from that of the acidic FSG. Our first efforts to test this possibility used fluorescein-conjugated histones, but were thwarted by the tendency of these proteins to adsorb onto cytoplasmic particles at the site of injection before they could diffuse to the intercellular bridges. We have now repeated the experiments with fluorescein-labelled lysozyme (FLy), a basic protein with an isoelectric point of 11.5, and obtained much clearer results. In 40 follicles the oocytes were injected with FLy and in every case fluorescence spread throughout the ooplasm, across the intercellular bridges and into the nurse cells (Fig. 2*a, c*). Fixation ranged from 1 to 2 h after micro-injection. In 25 additional follicles a nurse cell was injected and in no case did fluorescence spread to the oocyte (Fig. 2*b, d*), even after 2 h of incubation. FLy therefore crossed the bridges only in a direction opposite to that shown earlier for FSG.

An additional difference between FSG and FLy was seen in their capacities to move from nurse cell to nurse cell. In the earlier work FSG micro-injected into nurse cells moved directly to the oocyte, but was never seen to enter the other nurse cells,

Fig. 2 Fluorescence photomicrographs of follicles injected with proteins labelled with fluorescein isothiocyanate FITC by the method of Nairn¹⁵. Micro-injection was carried out hydrostatically as described earlier⁷. The follicles were incubated in blood from the animal diluted 1:2 with dissecting solution¹⁶. In dilutions as great as 1:10, blood protein concentration is still sufficient to maintain membrane permeability and steady-state potentials identical to those which occur in whole blood. Following injection and 1–2 h incubation, follicles were fixed either by freeze substitution (*a, c*) or in Carnoy's acid-alcohol (*b, d–h*), embedded in paraffin, sectioned and examined with a fluorescence microscope. Photographs were taken with Kodak Tri-X pan film (ASA 400). *a, c* Fluorescent lysozyme (FLy) micro-injected into oocytes; *b, d* FLy micro-injected into nurse cells. Note in *b* that protein has entered a co-bridged nurse cell (see Fig. 1), but failed to cross into the oocyte. *e, f* Methyl-carboxylated FLy (MCFLy) micro-injected into oocytes gave no detectable fluorescence in the nurse cells. *g, h* MCFLy micro-injected into nurse cells. Magnification: in all cases shown, here and in Fig. 3, the width of the nurse cell cap adjacent to the oocyte was about 450 μm .



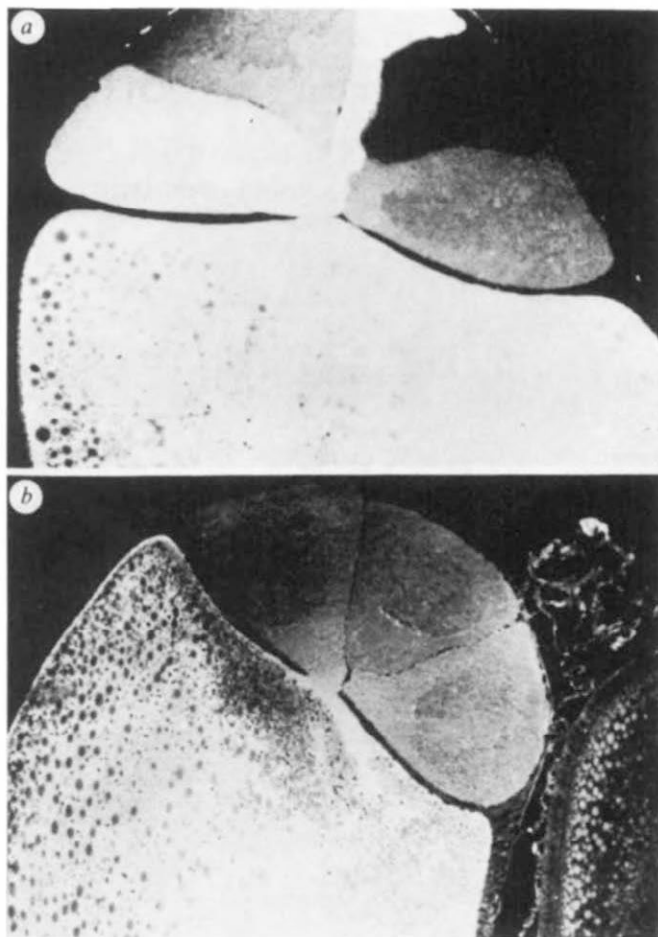


Fig. 3 *a*, Central nurse cell micro-injected with fluorescein-labelled haemoglobin. The protein has passed from the injected nurse cell into a co-bridged cell (lower left nurse cell), into the oocyte and from the oocyte to varying degrees into other nurse cells. *b*, An oocyte micro-injected with fluorescein-labelled myoglobin. A portion of an uninjected control follicle may be seen on the right. This neutral protein was found to migrate throughout the follicle whether oocyte or nurse cell was injected.

even when these were bridged directly to the injected cell (Fig. 1, centre quartet and right hand pair of nurse cells). Polarity apparently extends throughout the bridge complex, and whenever the negatively charged FSG entered this zone, it was unable to move in a direction away from the oocyte. By contrast, when nurse cells were injected with FLY, label entering the zone containing the bridge complex, although unable to proceed into the oocyte, was able to enter the nurse cells directly bridged to the injected cell (Fig. 2*b*). FLY thus seems to diffuse against the electrical potential gradient more readily than FSG.

To confirm that the differences in behaviour resulted entirely from the positive charge of lysozyme and not in part from its small size (molecular weight 14,600), the electrical properties of this protein were altered by methylcarboxylation of its ϵ -amino groups¹⁰. The polarity of lysozyme movement in the bridges was now completely reversed—it behaved exactly as FSG had behaved in the earlier work (Fig. 2*e-h*), passing from nurse cell to oocyte (48 injections), but not from nurse cell to nurse cell or from oocyte to nurse cell (40 injections). To extend the correlation between charge and mobility through the bridges, we also injected fluorescein-conjugated haemoglobin (21 injections) and myoglobin (28 injections), both of which have isoelectric points close to neutrality, and found that they were able to move in both directions across the bridges (Fig. 3*a, b*). When the fluorescein-labelled proteins were compared by electrophoresis in agarose plates at pH 7.0, they were found to exhibit the following sequence of mobilities towards the

cathode: lysozyme > haemoglobin = myoglobin > methylcarboxyllysozyme $\approx$ serum globulin. The charge dependence of the mobility of these proteins decisively supports the idea that the potential gradient across the bridges is steep enough to polarize the movement of soluble proteins.

Is the intracytoplasmic electrophoresis observed here a unique adaptation to the transport requirements of the oocyte-nurse cell syncytium or an exceptionally clear example of a more ubiquitous mechanism of polarity? The widespread occurrence of transcellular ion fluxes in developing cells^{1,11} suggests that intracytoplasmic potential differences are not unusual. The intercellular bridges, which are a special feature of our system, focus the potential gradient at the equator of the complex, and allow a qualitatively clear result in which fluorescent proteins either do or do not pass through to the uninjected cell. Although the diffusion of our fluorescent probes may prove not to be so dramatically restricted in polarized cells that lack an equatorial constriction, intercellular bridges occur more commonly than might be supposed. Many dividing cells generate transitory bridges of cytoplasm late in cytokinesis^{12,13}, and ionic communication through these channels attenuates gradually until division is complete¹⁴. Any dividing cell that is electrically polarized, therefore, should generate a transiently focused gradient in potential steep enough to restrict diffusion to the degree seen here for the oocyte-nurse cell syncytium.

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Corrigendum

In the *News and Views* item on 'Dendochronology' by Sara Champion, *Nature* **284**, 663 (1980), reference should have been included in paragraph four to the work of Hubert Polge and in particular to his paper, *Br. Arch. Rep.* **S51**, 77 (1978).

Erratum

In the letter 'Inhibin is absent from azoospermic semen of infertile men' by R. S. Scott and H. G. Burger, *Nature* **285**, 246-247 (1980), the following was omitted from line 13 on page 247 'relationship of inhibin concentrations to those of serum FSH in spontaneous disorders of the seminiferous epithelium has not been studied. It has been previously shown that a reciprocal'.

BOOK REVIEWS

What price conservation?

Kenneth Mellanby

WILDLIFE conservation is an important interest of a section of the British public — voluntary conservation organizations such as the Royal Society for the Protection of Birds, the Society for the Promotion of Nature Conservation and the many County Naturalists Trusts have several hundred thousand members. But the majority of the people of Britain are uninformed and indifferent. Is it therefore reasonable to expect wider support — and more money — from central government?

The British Nature Conservancy Council (NCC) is the official organization set up by Parliament "for the purpose of nature conservation and fostering the understanding thereof" and has the task of "maintaining the heritage of wildlife and natural features". Lack of funds and of popular support have always restricted the NCC in its work. During the financial year 1978–79 it received a grant from public funds of £9,535,000 of which £1,725,000 was earmarked for the purchase of the Ribble marshes, a wetland area of international importance for birdlife which would otherwise have been drained and used for intensive arable farming. Unfortunately this grant is unlikely to be increased or even maintained in value, particularly in the face of economic cut-back. As one way of gaining support, and in an attempt "to widen the public debate in nature conservation", the NCC asked Richard Mabey to make a special study of the place of nature in Britain. He was helped by the NCC, but was given a free hand to write his own book.

For some centuries Britain has been fortunate in producing gifted amateur naturalists who have often done more than the professional scientists to observe and record our wildlife and the changes in the countryside. Richard Mabey is already well known as a worthy recruit to this body of men and women, and for his books which include *Food for Free* (Collins: London, 1975) which describes the fruits, fungi and other delicacies which the observant may gather in our fields, woods and hedges, and *The Unofficial Countryside* (Collins: London, 1973) which deals with the city and suburbia, and shows how nature adapts to human interference, which may stimulate the spread of many species of

The Common Ground: A Place for Nature in Britain's Future? By Richard Mabey. Pp.280. (Hutchinson: London, 1980.) £8.95.

wild plants and animals. In *The Common Ground* he deals with human effects on what most people consider to be the natural countryside of Britain.

Of course Richard Mabey is well aware that almost all the landscape in Britain today has been affected by human activity, and that this situation, though changing year by year, has obtained for hundreds of years. Nevertheless, until recently, wild plants and animals were allowed to exist, often in greater numbers than were found in the dull and uniform forest which covered most of the country before agriculture changed the face of the land.

Here Richard Mabey deals at some length with old deciduous woods. Though they now only cover a fraction of the countryside, until relatively recently they continued, as a result of traditional management practice, to be beautiful, diverse and fascinating to the naturalist. They provided recreation for many and biological interest to the observant. Many such woods have now been felled and the land farmed, others have been transformed into uniform stands of exotic conifers.

In the same way farmland, with its hedges and shelterbelts, with old unimproved grassland and a multitude of ponds, allowed native species to flourish. In the past 30 years, farming has become much more productive, but the interest of the countryside and the abundance of wildlife in intensively farmed areas has greatly decreased. Hedges have been grubbed up, ponds filled and old grass with its wealth of wild flowers has been replaced with short-term leys of rye grass and clover.

The description of rural Britain in this book, based on its author's first-hand experience and his careful observation, is depressing to anyone with the slightest interest in natural history. Although only a few species of animals and plants have actually been exterminated, the numbers of

many others have been drastically reduced. The whole pattern and diversity of the landscape has been altered and impoverished. However, Richard Mabey is no elitist. He has no time for those who think that conservationists should concentrate their efforts on safeguarding a few reserves rich in rare species, where the public is excluded, while the whole of the rest of the countryside is sterilized. He clearly loves wildlife, and wants to share it with as many people as possible. To him the common species may be as valuable as the scarce, and he wants wildlife to be readily visible in all parts of rural Britain.

Though the NCC has a statutory duty to set up and maintain nature reserves, it fully agrees with Mabey that these are only a part of its responsibilities. Experience has shown that isolated reserves may be of decreasing importance, for they may lose the very species that they were set up to preserve. Thus Monks Wood National Nature Reserve, one of the few areas of old woodland in eastern England, has been known as a site supporting rare and local butterflies for over 150 years. These butterflies appeared to flourish in the nineteenth century when the wood was used for coppice and timber, and in the first part of the twentieth century when its main importance was as a pheasant shoot. Clear felling of much of the wood did not harm the Lepidoptera. Since 1953, when it became an official reserve, and has been managed primarily for wildlife, no fewer than 10 of the 14 species of butterfly most sought by entomologists have disappeared. This may partly be because of inefficient management; as I had this responsibility for part of the time I know that we still do much in ignorance of the results. But I think that the main reason is that a woodland reserve of 150 hectares (and, by our standards, this is a large lowland wood) is too small to support many insect populations if it is an island in an area from which exchange and reinforcement does not take place.

How then can we encourage the return of our wildlife, and how can we convince the public that this is something they should support? Richard Mabey is too honest to pretend that he has any easy answers. He sees the point of view of the farmer, and

does not pretend that conservation, or a return to the methods of previous centuries, will increase his income or his productivity. He makes a number of useful points about the way public and voluntary bodies can make minor contributions. His main suggestion is that "we need an imaginative land-use policy — a 'land ethic' — which recognizes that the fate of wildlife is inextricably connected with our own, and that nature conservation touches on the quality (and perhaps the continuance) of all our lives".

I agree strongly with his views on a land-use policy. Earlier this year, as the then chairman of the Environmental Division of the Institute of Biology, I organized a conference on this very subject. In it we discussed the various sectional demands for land — agriculture, forestry, water collection, recreation, waste disposal, nature conservation. Conservationists were agreed that we need to find some way of giving priority to wildlife even when economic forces work in the opposite direction. The difficulty is to find a reason for wildlife conservation which is convincing to those not already committed.

While I fully agree with Richard Mabey that wildlife may contribute greatly to the quality of life — at least of my own life, as one much involved in this field — I am doubtful whether I can support the idea that human continuance on the globe is in any way involved. I was equally worried by the recent *World Conservation Strategy*, produced by the International Union for Conservation of Nature and Natural Resources (see *Nature* 284, 113; 1980), which relates wildlife conservation to sustainable development. I fear that conservationists may sometimes claim too much. On a world scale it may be wise to try to preserve all species, particularly plants which may have an unexpected potential or contain genetic material which may contribute to better cultivars of food species. I personally would be very upset if the large wild mammals of Africa and Asia were exterminated, but were I a peasant farmer whose crops were consumed by elephants and whose stock was killed by lions I would welcome the elimination of animals which, to me, were pests. British farmers have only gained from the extinction of the wolf, the bear and the beaver, however much some conservationists would welcome the reintroduction of these species.

The modern arable farm in Britain, which has done so much to harm wildlife, has not suffered in the process. The land is producing higher yields, and by any reasonable measure the fertility of the soil is being steadily improved. The disasters arising from monoculture, reduced rotations and increased fertilizer use which some ecologists foretold have not been apparent, though wildlife (including weeds and pests) has been devastated. I am unable to advance any purely economic reason

when I try to persuade a farmer to take active steps to encourage wildlife. I have to rely on aesthetic, scientific or even spiritual values — in fact on the 'quality of life'.

Fortunately many farmers are willing to set aside small patches of land where cover may develop and wildlife survive. This is good conservation, but it makes no contribution to agricultural efficiency, in fact it probably reduces productivity slightly. This is the sort of compromise which the late Ivor Brown described as the British genius for making the second best of both worlds.

For, from the point of view of wildlife, it is only a second-best solution. If we are really serious, we must be prepared to establish far more and far larger nature reserves, even sacrificing good agricultural land for this purpose. We must also

persuade farmers and foresters to make real sacrifices in productivity. Perhaps *The Common Ground* will help to produce a climate of opinion where such developments are possible. In any event it is an excellent book which will make its readers, even those within the conservation movement, better informed. By showing them how serious have been our losses, it should encourage them to redouble their efforts to protect our wildlife. Whether it will appeal to the non-caring majority is another question. □

Kenneth Mellanby was employed by the Nature Conservancy from 1961 to 1973, and was the first director of Monks Wood Experimental Station. He is President of the Bedfordshire and Huntingdonshire Naturalists Trust.

World of the stellar life-cycle

J. D. Barrow

White Dwarfs, Black Holes: An Introduction to Relativistic Astrophysics. By R. Sexl and H. Sexl. Pp. 198. (Academic: New York and London, 1979.) \$13.50, £7.60.

STARS often die very violent deaths and their mortal nature has caused modern astronomers to focus more and more attention upon the circumstances and consequences of their demise. In so doing, astronomers have become astrophysicists. Supernovae, pulsars, quasars, black holes and the entire body of high-energy cosmic phenomena require for their elucidation a delicate amalgam of knowledge concerning nuclear and quantum physics tempered by an understanding of the all-encompassing effects of gravity.

The more massive a star, the higher the temperature and rate of nuclear ignition in its central regions, the faster it evolves and the more dramatic its death throes. After their nuclear fuel is exhausted the most massive stars collapse; at first slowly but then catastrophically. A huge explosion can transform this unstable stellar state into a supernova explosion, expelling the outer layers of the star and scattering heavy elements and high-energy particles out into space in dramatically visible fashion.

Our Sun is not in the heavyweight stellar league. A member of the stellar bourgeois, it awaits a slower and quieter death. Although at present it burns only about 10^{16} of its 10^{30} kg of fuel every day, eventually, like all stars, it will suffer an irresolvable energy crisis. When the internal supply of fusible hydrogen is exhausted, exothermic fusion of other heavier elements must be employed to stave

off the pressures of gravitational collapse. Gravitational potential energy can be tapped by adjusting to a more stable configuration with a contracted core and extended outer regions. The star inflates and appears superficially cooler and redder; it has become a 'red giant'. In such a bloated state our Sun would have expanded to encompass the orbit of Mars.

As the central temperature rises inexorably, heavier and heavier elements undergo exothermic nucleosynthesis until a turning point is reached: even if the temperature is high enough for the fusion of elements heavier than iron, these fusion processes are endothermic. The star has now lost its conventional means of pressure support.

The smallest stars, with mass less than ~ 1.5 that of the Sun, can form a stable, compact state termed a 'white dwarf'. To reach such a configuration the Sun would have to shrink one-million-fold to a size comparable with the Earth and, in so doing, increase its average density to about 10^3 kg per cubic centimetre. It would sit securely locked between the opposing forces of gravity and electron degeneracy pressure. The reason gravity can be countered is the exclusive nature of subatomic society. No two particles of a type that includes neutrons, protons and electrons can occupy the same energy state. In cool 'laboratory' gases pressure is determined by the kinetic energy of the constituent molecules and energy can be extracted by dropping an atomic electron into a lower energy orbit. In degenerate material the energy niches fill from the bottom upwards and no low-energy slots are left vacant. The density, rather than the temperature, determines the kinetic energy of the electrons. Once in the white dwarf state a dying star can only cool to a dark ember. Such are the non-linearities of gravity that, the larger a star in its youth, the smaller the dwarf it becomes in death.

If a star exhausts its nuclear fuel when a little larger than 1.5 Suns, its internal

stresses can become so large that electrons are eventually torn from their atomic orbits and crushed into the nuclei. The result is a closely packed lattice of about 10^{57} neutrons, squashed to a nuclear density one-billion times higher than within a white dwarf. A strongly repulsive degeneracy pressure now arises between the neutrons and the squeezing is halted, eventually leaving a 'neutron star' in stable equilibrium, just a few kilometres in diameter. Free neutrons decay into protons and electrons in about ten minutes but the neutrons in a neutron star, like those comprising an atomic nucleus, do not undergo such decay. The resulting proton would have to occupy an extremely unfavourable energy state.

White dwarfs and neutron stars have been observed by radio, X- and gamma-ray astronomers and rapidly rotating neutron stars provide an explanation for the 'pulsar' phenomenon.

The fate of the heaviest stars is well known to the average reader: unstoppable collapse to a 'black hole' state of ever-increasing density. A point of no return, the 'event horizon', is encountered by infalling material and no known forces can support the star once it has been encompassed by this surface. The collapse pro-

ceeds inevitably to an unknown singular state.

This is the exotic life-cycle of stellar bodies and its documentation often requires a description of matter in gravitational fields too strong for Newton's classical theory to be accurate. And this is the world that the Sexls equip the student to explore. *White Dwarfs, Black Holes* is a translation of a new edition of their successful German text explaining the nature of general relativity, gravitational waves, relativistic astrophysics and elementary cosmology. It is clear and concise, didactic in style and eminently suitable for final-year undergraduates in physics or astronomy. General relativity is described in qualitative terms (no prior familiarity is assumed and no tensor analysis is developed in the text) with a good description of its experimental status although, unfortunately, the observations of the binary pulsar are not included. The authors maintain a good balance between theory and observation by concentrating upon hard, testable results rather than speculative possibilities. However, it is odd to see some of the theory of primordial nucleosynthesis developed without a glimpse at the observational data on cosmic abundances of very light elements. The

authors use physical reasoning wherever possible and avoid the use of inessential mathematics. SI units are used throughout and illustrations are skilfully employed to augment, rather than merely re-state, the textual material. A series of exercises are scattered through the text and many have 'solutions' provided. Some of these questions are a little too open-ended to be useful for private study because specific background references are not provided.

Unfortunately referencing is often scanty and imprecise. There is a considerable amount of name-dropping in the text without reference to sources or follow-up articles, and the bibliography is categorized in a fashion that may be too general for students. A list of specific semi-popular articles would have been useful here. But these are second-order problems in a book that is clearly written, well produced and realistically priced. I recommend it to any student who is contemplating graduate work in astrophysics, to teachers seeking a well-ordered foundation course, or indeed to anyone curious to know what relativistic astrophysics is about. □

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Experts on vacua

John Yarwood

Methods of Experimental Physics. Volume 14, Vacuum Physics and Technology. Edited by G.L. Weissler and R.W. Carlson. Pp. 593. (Academic: New York and London, 1979.) \$55, £13.80.

THIS BOOK contains a great deal of useful information, concisely and well-written by a number of American authors, all of whom are widely experienced in vacuum science and technology and have up-to-date knowledge gained in academic or industrial research.

The difficulty in judging the book is to assess at whom it is aimed. Despite the fact that the purpose (according to the title of this series) is experimental, a fairly sophisticated mathematical notation is almost always used by the authors so that a reader of that useful type of person — the technician — is, by and large, ruled out. The style is really more suited to the post-graduate student of science or technology.

Roughly the first half of the text is concerned with the production and measurement of vacuum (mostly high and ultrahigh). Although the authors frequently give ideas and explanations which are novel, the topics concerned have received much attention in textbooks and journals over the past 30 years or so. One wonders, therefore, whether it wouldn't have been preferable to omit most of the

well-worn material and concentrate on the more novel second half, beginning with "Metal Vacuum Systems and Components". Here, modern information is provided about components and materials and their most effective use in the design of vacuum systems for specific applications.

The book contains no information about how the undergraduate student should go about undertaking practical work in vacuum science and technology.

However, it will be most useful to the member of academic staff who is concerned with the development of such practical experiments and wishes to write them up satisfactorily for undergraduates, students beginning research and for special short courses. □

John Yarwood retired in 1978 as Head of Physics at the Polytechnic of Central London, UK, and is the editor of Vacuum.

First of a series on inflammation

G. P. Lewis

Handbook of Inflammation. Volume 1, Chemical Messengers of the Inflammatory Process. Edited by J. C. Houck. Pp. 422. (Elsevier/North-Holland: Amsterdam and New York, 1979.) \$75, Dfl. 154.

IN THE introduction to this first volume of the *Handbook of Inflammation*, the editor, John C. Houck, gives a splendid though brief account of the various steps in the inflammatory process. The book has then been arranged so that the mediators connected with each stage have been discussed.

Our old friend histamine comes first, but brought up to date in a very interesting way. In addition to the work of recent years on H_2 receptor-mediated actions, the

author reviews current ideas on the possible regulatory function of histamine on activities of inflammatory cells. This theme of cell-cell interactions mediated by soluble mediators is taken up again later in the book in several chapters. Lymphokines, which were originally thought only to be the soluble products of activated T-lymphocytes, have now been shown to be not strictly restricted to immune activation pathways. The author of this particular chapter points out that release of soluble intracellular mediators might be a basic method of biological communication and describes lymphokines and cytokines as a plethora of inflammatory mediators which even includes viral and immune interferon.

The kinin system is described in some detail with the emphasis on biochemical aspects; only two of the 46 pages cover the role of kinins in inflammation. The review very adequately covers the relationships between the kinin and fibrinolytic systems and Hagemann factor, and the repetition

of much of this in a later chapter on Hagemann factor seems to be unnecessary.

There have been so many reviews on prostaglandins in the past year or so that I was pleased to see only one modest chapter on this subject. But with the increasing knowledge of the importance of products of arachidonic acid metabolism other than prostaglandins, it seems unfortunate that this chapter did not include more information about thromboxane and the lipoxygenase products including SRS-A.

The book includes a very comprehensive chapter on complement and chemotaxis in which old and new methodologies in this complicated field are explained and compared.

In addition to discussions on mediators which might affect intracellular activities, there are two accounts of some cellular components which might be affected and which might play a role in the tissue damage which accompanies chronic inflammatory conditions. These are the lysosomal enzymes of which we are told there are more than 40 which are mostly hydrolytic and the acid cathepsins. They might well play a role not only in causing tissue damage but also in the formation of biologically active degradation products.

But in spite of an enormous amount of research in this field, the extent of the involvement of these enzymes is not known. However, the discussions include an interesting description of the control mechanisms involved which might give a pointer to the direction that future work should take.

Volume 1 concludes with three fascinating chapters about factors controlling the growth of cells. Many of us who have become familiar with the mediators of the vascular changes and cell migration and activation in inflammation are not so familiar with growth factors and their inhibitors. The significance of macrophage growth factor in biology, for example, is just beginning to emerge. The future will reveal how important are the factors controlling differentiated cell populations at sites of inflammation and their role in inflammation and tissue repair.

Not only is the proliferation of inflammatory cells a significant aspect of inflammation but the proliferation of endothelial cells in growing blood vessels is also important, particularly in tissue repair. The editors decided to cover this aspect with a review on tumour angiogenesis which I personally found

exciting and stimulating.

Finally we are treated to another fascinating account, this time about chalcones, the tissue-specific inhibitors of cell proliferation. The chapter starts with the story of the early theoretical considerations and the early experimental evidence for the existence of chalcones, and goes on to describe the arguments of mitotic stimulators versus mitotic inhibitors. A final glimpse into the future suggests the possibility of inhibiting the mast cell proliferation in *Urticaria pigmentosa* with a mast cell chalone.

There is no doubt that this first volume of the *Handbook* is a valuable addition to the literature on inflammation. It is a pity that the book, superficially well produced, has suffered from deplorable copy-editing and proof-reading — over 200 typographical and grammatical errors, and discrepancies in the references, have been noted in just two chapters. A list of these has been sent to the publishers, who will hopefully take greater care over subsequent volumes. □

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Science from the Serengeti

Hans Kruuk

Serengeti: Dynamics of an Ecosystem. Edited by A.R.E. Sinclair and M. Norton-Griffiths. Pp.389. (University of Chicago Press: Chicago and London, 1980.) \$28.50, £17.10.

THE STREAM of papers emanating from the once so active Serengeti Research Institute has dwindled over the past decade, and the Institute is now only a shadow of its former self. The large concerted effort by scientists from many disciplines to provide an ecological basis to the management of this huge ecosystem in Tanzania has virtually stopped. What is the result of all this effort? *Serengeti* is a sample of the diversity of output of the Serengeti Research Institute, with contributions from a dozen of its former scientists; the book is an attempt to take stock, and to evaluate some of the research in terms of conservation and management. It is clearly focused on the phenomenon for which the Serengeti is justly famous, the vast population of migratory ungulates.

Of the 13 chapters, three by A.R.E. Sinclair describe the Serengeti background, the history and the migrations of its animal populations; the interactions between the most important migratory and resident ungulates and the vegetation are studied in detail in a further four chapters

by S.J. MacNaughton, P.J. Jarman and Sinclair, L. Maddock, and M. Norton-Griffiths. C.J. Pennycuik analyses the cost of locomotion to the migrants, R. Hilborn and Sinclair describe simulation studies of the Serengeti populations, and P.J. and M.V. Jarman compare ungulate social organizations. Three chapters, one by B.C.R. Bertram, one by J.P. Hanby and J.D. Bygott, and one by D.C. Houston look at the large predators and scavengers, their social systems, population changes and adaptations to the herbivore migrations.

The book is more a collection of distinct individual contributions than a united evaluation. However some of these contributions are outstanding; I was particularly struck by MacNaughton's beautiful experimental approach to the grazing relationships. Norton-Griffiths' clear analysis of the relation between animal populations and movements, climate, fire and other factors in this huge ecosystem should be an example to many other studies in this field. The predator chapters give a summary of much of the work that has been done on the Serengeti carnivores, and they indicate once again the complicated consequences for the carnivores' social organization of a rapidly increasing prey population. At the end of the book there is a useful list of over 300 references to the scientific publications of the Research Institute.

In general, the quality of all the chapters is good, and the book is full of fascinating observations which more than overshadow its shortcomings. One feels the absence of a chapter on the botanical and soil studies

carried out in the Serengeti — everything is seen through zoologists' eyes. The two introductory chapters with their categorical statements fall somewhere between reviews and new contributions, without doing full justice to either of these functions. Scientific modesty would be served if it were pointed out that present population levels of ungulates are well above anything that was predicted as 'equilibrium' only a few years ago, thus indicating some of the limitations of the work of ecologists in such an ecosystem, even when the most elegant predictive models are used. And clearly it is prudent not to attempt to explain these population increases only in terms of the eradication of rinderpest in the early 1960s, since non-vulnerable species also increased — a fact conveniently forgotten in some of the chapters.

These are relatively minor grumbles, however, as are the irritation of the printing errors and the unaccredited photographs in one batch in the middle of the book which are not related to the text. What I missed most of all was a final chapter by the editors in which they could have pulled the strings together instead of leaving the papers as separate entities.

Despite all this, the book stands as an important monument to the efforts of the Serengeti Research Institute, and I have no doubt that it will be a work of reference for many studies elsewhere. I hope that before long the Institute itself will again be in a position to make full use of it. □

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Microwave spectroscopy

H.W. Kroto

Modern Aspects of Microwave Spectroscopy. Edited by G.W. Chantry. Pp. 513. (Academic: London and New York, 1979.) £38.80, \$89.50.

YET another expensive volume containing articles by several authors! I have never been totally convinced that this is a satisfactory way to publish reviews. Such books often suffer from one or more of three main defects: the material tends to be three or four years out of date on publication, the various chapters may be uneven in quality and balance, and finally, more often than not, many chapters are intrinsically re-hashes of previously published reviews, often by the same authors. On the whole this book is better than average on these three scores and, indeed, microwave spectroscopy is one of the few areas for which there does not exist a surfeit of such books.

The first chapter, "Microwave Spectrometers" (G.W. Chantry and G. Roussy), gives rather brief general descriptions of various experimental techniques which are used to carry out microwave spectroscopy. Although it gives a useful overview I felt the chapter was too short to cover microwave hardware in sufficient depth. The technical aspects are important as microwave spectrometers are no longer commercially available. The chapter has a comprehensive compilation of references.

The second and third chapters are entitled "Microwave-Microwave Double Resonance" (J.G. Baker) and "Infra Red-Microwave Double Resonance Techniques" (H. Jones), respectively. Both are very useful, detailed reviews of the double-resonance method. They complement each other well and are presented at about the right level. The latter includes a section on optical-microwave experiments and so the two chapters, taken together, give a quite comprehensive survey of a technique which has revolutionized high-resolution molecular spectroscopy.

The fourth chapter on modern submillimetre microwave scanning spectroscopy, by A.F. Krupnov, highlights recent advances in the automation of the beautiful wide-band millimetre wave scanning spectroscopy technique pioneered by this author. The techniques described here would be useful were they available to the scientific community. However, the direct experimental value of this chapter is limited because the necessary high-power microwave sources appear only to be available in the military installation at Gorki in the USSR.

Chapter 5, by J.W. Fleming, deals with interferometric spectroscopy at millimetre and submillimetre wavelengths. Although

this is a useful review of the technique, I find it a curious choice for this collection, though it does give a welcome change of pace. I suspect that the audience for the other articles is biased towards higher resolution techniques and that this review may not be readily found by those to whom it would be most valuable.

The last contribution is yet another review on interstellar molecules, entitled "Astrophysics of Interstellar Molecules" (G. Winnewisser, E. Churchwell and C.M. Walmsley), and it is valid to ask whether another review is justified. This one is. It

presents a different perspective as it is more strongly biased than previous reviews towards spectroscopy — as is appropriate for this volume. It is well balanced and covers the appropriate material comprehensively from a microwave spectroscopists point of view. In addition it is up to date with numerous useful tables.

In all, this is a timely volume for the practising microwave spectroscopist. □

H.W. Kroto is a Reader in Chemistry in the School of Molecular Sciences, University of Sussex, Brighton, UK.

A weather controversy

M.W. Moncrieff

Weather Modification. By G. Breuer. Pp. 178. (Cambridge University Press: Cambridge, UK, and New York, 1980.) Hardback £10.50; paperback £3.75.

WEATHER modification is an emotive subject among meteorologists, and over the past few decades there has been a great deal of controversy. The physics and dynamics of atmospheric phenomena are more complex than most physical systems and, more often than not, incompletely understood. However, the potentially significant social, agricultural and economic rewards of successful weather modification have ensured that a considerable amount of financial backing has been available for experiments, often in the absence of sufficient scientific knowledge. The 'seeding' of clouds by silver iodide crystals to enhance rainfall is one example of how a laboratory technique was applied to real atmospheric clouds before a quantitative knowledge of the physics and dynamics of these systems was available. This book illustrates the basic gulf between the practical application of simple techniques to control complex dynamical systems, with a considerable number of commercial, political and economic consequences, and the background of insufficient theoretical understanding. It is no surprise that debate exists with such contrasting motivations surrounding the subject.

The book, in three chapters, is generally well written and is informative to the non-specialist, and the above-mentioned gulf between theory and application is well documented. It is not intended for the specialist, who might be irritated by the large amount of speculative science and continual reference to political and military application; this is especially true of the last chapter. However, the extensive bibliography is useful to both specialist and non-specialist. In general, a balanced viewpoint on a highly controversial topic is

retained, and the layman should obtain much useful information.

The first chapter provides a background largely dealing with the philosophy and history of the topic, and brings out the controversies of the subject, particularly that related to cloud seeding. The introduction to the basic physics is unfortunately rudimentary and out of date. The reader may be left with the mistaken impression of cloud physics and dynamics being a very inexact science; recent advances in computational and mathematical techniques are not considered and the cloud models are typical of those seen in school textbooks. An important aspect stressed is the inadequate statistical significance tests in most cloud-seeding experiments, recognized as a major criticism of the subject in general.

The second chapter is undoubtedly the most quantitative and scientific; subjects such as fog dispersal, seeding of convective and orographic clouds, hail and lightning suppression, and the modification of hurricanes are discussed. The author covers experiments in many countries, mainly the USA, USSR and Australia. An interesting historical review of the basis of cloud-seeding, beginning with Langmuir's first laboratory experiments and the initial euphoric and extravagant claims made about weather control, is included. The seeding of cumulus clouds is reviewed in some detail, but the seeding of orographic clouds has probably been the most successful and has the best history of controlled scientific experiment, especially in the USA. It is no surprise that the modification of hurricanes has achieved little success since the basic dynamics, particularly in the growing stages, are not well understood. The most speculative discussion is centred on the modification of large-scale weather systems and climatic change. This has not yet been deliberately attempted, although man-made effects, such as carbon dioxide production due to industrialization, affect climate although their importance is debatable. The possible causes of climatic change are not reviewed in any detail, but these are also beyond the scope of the book. The economic and political interests in weather modification, particularly in cloud seeding, are des-

cribed. The importance of the financial support by these interests is probably considerable; scientific support in the subject is, by contrast, largely a by-product of more fundamental research.

The last chapter is mainly speculative and qualitatively deals with social, economic and legal aspects; this chapter is more suitable for the non-scientist. I feel that it is somewhat laboured, could have been condensed considerably and is something of an anticlimax. The importance of

military and political interests in research and experiment are again brought out, and perhaps this, above all, emphasizes the lack of proportion in the weather-modification picture. Indeed, it is not clear from this book that the present approach to weather modification is any more scientific than the original experiments in cloud seeding. □

M. W. Moncrieff is a Lecturer in Atmospheric Physics at Imperial College, University of London, UK.

Rats in the lab

Maurice Smith

The Laboratory Rat. Volume I, Biology and Diseases. Edited by H.J. Baker, J. Russell Lindsay and S.H. Weisbroth. Pp. 448. (Academic: New York and London, 1979.) \$52.50, £29.40.

THIS is the first of two volumes on the laboratory rat, following earlier publications on the laboratory rabbit and guinea pig which were also sponsored by the American College of Laboratory Animal Medicine.

Contributions from 24 authors have been brought together in 15 chapters and two appendices, forming a most comprehensive reference work. The success of the editors in consolidating the technical material available has been balanced by their equivalent success in producing a readable and yet authoritative work which should be available for reference by almost any worker using laboratory bred rats. This is best illustrated by the first chapter which describes in great detail and clarity the origin of the major outbred strains and the early development of inbred strains.

That this is a mainly American story is some reflection of the earlier recognition by the American research world of the need for defined laboratory animals. However it is significant that despite the understandable selection of authors mainly from the USA, those asked to write the chapters on taxonomy, genetics and inbred strains were British, which is due recognition for the work done in Britain on this aspect of laboratory animal science.

The biological chapters in the first half of the book cover genetics, anatomy, physiology, haematology, clinical biochemistry, nutrition, feeding and housing. They show a refreshing recognition of the relationship between biological and experimental variables, which is sometimes not adequately recognized by those using animals as experimental tools.

One of the best examples of this approach is Chapter 8, "Housing to Control Research Variables", which replaces what could have been a standard chapter on housing with a consideration of the effects of physical, chemical and micro-

bial factors on biological responses.

The chapters on disease are without exception well done and each may be considered a definitive text in its own right, especially when supported by the very comprehensive references which are a characteristic of the book as a whole.

There are, however, occasional anomalies which could cause misunderstanding among British readers. Examples of this are the description of *Corynebacterium kutscheri* as the causative agent of pseudotuberculosis, which in the UK is usually attributed to *Yersinia pseudotuberculosis*. Also, at no point is the term chronic respiratory disease (which is generally recognized in the UK) used in reference to respiratory infection. There is a tendency to deal with respiratory infections as quite separate conditions, and the mixed nature of the outbreaks of respiratory disease so commonly found in conventional rat colonies is not sufficiently emphasized.

It is particularly gratifying that chapters on neoplasia and lesions associated with ageing have been included, while the chapter on human health hazards will prove very useful to those involved with safety problems in animal units.

The appendices on drug dosage and selected normative data are welcome and useful additions. It is to be hoped that some consideration may be given to the enlargement of these sections in future editions.

The book is profusely illustrated throughout with black and white photographs of excellent quality together with more-than-adequate tables, diagrams and figures. Understandably, colour has not been used, but despite this the quality of the publication with its strong hard-backed binding is of the high standard one has come to expect from ACLAM.

Volume I of *The Laboratory Rat* is without doubt a timely and very welcome addition to the literature. I await Volume II, *Research Applications*, with considerable interest and with the certain knowledge that *The Laboratory Rat* will become the standard work of reference on this subject. □

Maurice Smith is Director of Central Animal Services at the University of Cambridge, UK, and Laboratory Animal Consultant to the Open University, Milton Keynes, UK.

Reproduction in perspective

Barry Cross

Oxford Reviews of Reproductive Biology. Volume 1, 1979. Edited by C.A. Finn. Pp. 485. (Clarendon/Oxford University Press: Oxford and New York, 1979.) £30.

UNLIKE several biological disciplines of recent origin, the biology of reproduction has cast its spell on enquiring man for centuries, if not millenia. Even so the acceleration of activity in the last three decades has been remarkable, with research proliferating in the new universities and colleges as well as in long-established academic institutions. Whereas 40 years ago reproductive research was almost exclusively a male preserve, the recession of sex discrimination has brought a tide of female workers to reproduction laboratories, much to the advantage of the subject. Dr Anne McLaren FRS is the chairman of the editorial board of this annual review series and two of the chapters in the first volume are written by talented women scientists.

The book contains seven reviews of uniformly high quality, clearly written and appropriately comprehensive. The authors, who are all at the height of their investigative careers, do not skimp, plagiarize or patronize the reader. They dig deep into the foundation of their respective subjects often referring to much earlier work, and critically examine much present-day dogma. The mix is certainly not what one would expect to find in a textbook on the subject or in an introduction for the educated layman. It perhaps most obviously reflects the impact of refinements in chemical techniques on physiological problems. We have authoritative discussions on the current status of relaxin (D. G. Porter), uterine proteins (R. J. Aitken), molecular biology of spermatogenesis (A.R. Bellvé) and preservation of semen (P. F. Watson), as well as on the established favourites — sexual differentiation of the brain (J. E. Booth), control of gonadotrophins (C. A. Wilson) and milk secretion (R. C. Richards). All are treated in a refreshing way and make stimulating as well as informative reading.

The text is efficiently edited by Professor C.A. Finn and contains very few misprints. Some readers may feel that the scarcity of figures is a disadvantage and that the quality of some of the micrographs in the plates at the end of the book is not very impressive.

But these are small defects, and annual reviews in the series will be eagerly awaited.

Barry Cross is Director of the ARC Institute of Animal Physiology, Babraham, Cambridge, UK, and Head of its Neuroendocrine Group.

BOOKS RECEIVED

Computer science

- CRONHJORT, B. (ed.) *Real Time Programming 1978. Proceedings of the IFAC/IFIP Workshop Mariehamn/Åland, Finland*. Pp. viii + 129. ISBN-0-08-024492-0. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1980.) \$22.50 £10.50.
- NOVAK, M. (ed.) *Software for Computer Control. Proceedings of the Second IFAC/IFIP Symposium on Software for Computer Control, Prague, Czechoslovakia*. Pp. xii + 420. ISBN-0-08-024448-3. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$73.00.
- SWARTZLANDER, E.E. jr. (ed.) *Computer Arithmetic. Benchmark Papers in Electrical Engineering and Computer Science/21*. Pp. xiii + 378. ISBN-0-87933-350-2. (Stroudsburg, Penn.: Dowden, Hutchinson & Ross, Inc. 1979.) Distributed world wide by Academic Press, \$45.00.
- THE ELECTRICAL RESEARCH ASSOCIATION LTD. *The Engineering of Microprocessor Systems. Guidelines on System Development*. Pp. xii + 175. ISBN-0-08-025435-7. Hardback ISBN-0-08-025434-9 flexi. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$15.00 £7.50 Hardback \$6.00 £2.95 flexi.

Applied biology

- AYRES, J.C., MUNDT, J.O. and SANDINE, E. *Microbiology of Foods*. Pp. 708. ISBN 0-7167-1049-8 (Freeman: Oxford, 1980.) £10.80.
- BRIGGS, M. H. and CORBIN, A. (eds) *Advances in Steroid Biochemistry & Pharmacology Vol. 7*. Pp. 125. ISBN 0-12-037507-9 (Academic: London and New York, 1980.) \$23.00; £9.80.
- BROWN, K. and COOPER, S. J. (eds) *Chemical Influences on Behaviour*. Pp. 670. ISBN 0-12-136780-0. (Academic: London and New York, 1979.) £39.20; \$90.50.
- BROWN, V.K. *Acute Toxicity: In Theory and Practice*. Pp. 159. ISBN 0-471-27690-1. (John Wiley & Sons: Chichester, 1980.) £10.00.
- CAMPBELL, P. N. and MARSHALL, R. D. (eds) *Essays in Biochemistry*, vol. 15. Pp. 130. ISBN 0-12-158115-2. (Academic Press: Published for Biochemical Society, 1979.) \$11.50; £4.80.
- CROMPTON, C. R. *Additive Migration from Plastics into Food*. Pp. ix + 234. ISBN-0-08-022465-2. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1980.) \$47.00 £21.00.
- GALJAARD, H. *Genetic Metabolic Diseases: Early Diagnosis and Prenatal Analysis*. Pp. xvi + 880. ISBN 0-444-80143-X (Elsevier/North-Holland: Amsterdam, 1980.) Dfl. 285.00; US\$ 139.25.
- GIBSON, J. E. *Thin Shells; Computing and Theory. Structures and Solid Body Mechanics series*. Pp. ix + 289. ISBN-0-08-023275-2 hardback ISBN-0-08-034204 flexi. (Oxford, New York: Pergamon, 1979.) \$30.00 £15.00 hardback \$15.00 £7.50 flexi.
- HARWOOD, R. F. and JAMES, M. T. *Entomology in Human and Animal Health*. Seventh edition. Pp. vi + 548. ISBN-0-02-451600-3. (New York: Macmillan Publishing Co., Inc.; Toronto: Collier Macmillan Canada, Ltd.; London: Baillière Tindall, 1980.) £18.00.
- HECHT, S.M. (ed.) *Bleomycin: Clinical, Biochemical, and Biological Aspects*. Pp. 351. ISBN 3-540-90395-X. (Springer: Berlin, Heidelberg and New York, 1979.) DM 79.00; \$34.50.
- JANTSCH, E. *The Self-Organizing Universe. Scientific and Human Implications of the Emerging Paradigm of Evolution. Systems Science and World Order Library*. Pp. xvii + 343. ISBN-0-08-024312-6 hardback ISBN-0-08-024311-8 flexi. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$44.00 £20.00 hardback \$15.00 £6.65 flexi.
- JORGENSEN, S. E. *Lake Management. Water Development, Supply and Management*. Pp. xii + 167. ISBN-0-08-022432-6. (Oxford, New York: Pergamon, 1980.) \$36.00 £15.50.
- NRIAGU, J. O. (ed.) *The Biogeochemistry of Mercury in the Environment. Topics in Environmental Health, Volume 3*. Pp. xv + 696. ISBN-0-444-80110-3. (Amsterdam, New York, Oxford: Elsevier/North-Holland Biomedical Press, 1979.) US \$136.50 Dfl. 280.00.
- OUCHTERLONY, O. and HOLMGREN, J. (eds) *Cholera and Related Diarrheas Molecular Aspects of a Global Health Problem: 43rd Nobel Symposium co-sponsored by WHO*. Pp. 246. ISBN 0079-645X (S. Karger: Basel, 1980.) Sfr. 115.; DM 138; \$69.00.
- PARRY, D.A.D. and CREAMER, L.K. (eds) *Fibrous Proteins: Scientific, Industrial and Medical Aspects*. Vol. 1. Pp. 508. ISBN 0-12-545601-4. (Academic: London and New York, 1980.) £19.80; \$46.00.
- PARRY, D.A.D. and CREAMER, L. K. (eds) *Fibrous Proteins: Scientific, Industrial and Medical Aspects*. Vol. 2. Pp. 257. ISBN 0-12-545602-2. (Academic: London and New York, 1980.) £13.00; \$30.00.
- PLIMPTON, P. A. (ed.) *Jottings of a Harvard Botanist*. Pp. 401. ISBN 76-52949 (Harvard University Press; Cambridge, Massachusetts and London, 1980.) £7.80.
- PLOMIN, DEFRIES and McCLEAN. *Behavioural Genetics: A Primer*. Pp. 417. ISBN 0-7167-1128-1. (Freeman: San Francisco, 1980.) \$15.00 Flexi; \$25.00 Hardback.
- POLLARA, B., PICKERING, R.J., MEUWISSEN H.J. and PORTER, I.H. (eds) *Inborn Errors of Specific Immunity*. Pp. 469. ISBN 0-12-559650-2 (Academic: New York and London, 1980.) \$34.00.
- POLLOCK, J. R. A. (ed.) *Brewing Science Vol. 1. Food Science and Technology: A Series of Monographs*. Pp. 604. ISBN 0-12-561001-7. (Academic: London and New York, 1979.) £33.00; \$76.00.
- RICHARDS, V. (ed.) *Current Cancer Immunology. Progress in Experimental Tumour Research*. Volume 25. Pp. xii + 296. ISBN 3-8055-3033-1. (Basel, München, Paris, London, New York, Sydney: S. Karger, 1980.) Sfr. 145 DM 174 approx. US \$87.00.
- SMITH, G. (ed.) *Proceedings of the Sixth International Congress on Hyperbaric Medicine. University of Aberdeen, Scotland*. 1977. Pp. xii + 468. ISBN-0-08-024918-3. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$50.00 £25.00.
- SUMMERFIELD, R. J. and A. H. BUNTING. *Advances in Legume Science*. Vol. 1 of the *Proceedings of the International Legume Conference Kew, 31st July-4th August 1978*. Pp. 667. ISBN 0-85521-223-3 (Royal Botanic Gardens: Richmond, 1980.) £15.00.
- THYS, J.P., KLASTERSKY, J. and YOURASOWSKY, E. (eds) *Aerobic Gram-Negative Bronchopneumonias. Proceedings of the Symposium on Aerobic Gram-Negative Bronchopneumonias, Brussels, September, 1978*. Pp. 183. ISBN-0-08-025333-7. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1980.) \$25.00 £11.00.
- UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE. *Problems of the Agricultural Development of Less-favoured Areas in Europe. Proceedings of a Symposium of the Committee on Agricultural Problems Economic Commission for Europe and Food and Agriculture Organisation on 22-26 May, 1978*. Pp. xxi + 252. ISBN-0-08-024456-4. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$35.00 £17.50.
- UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE. *Energy Aspects of the Forest Industries. Proceedings of a Seminar organized by the Timber Committee of the United Nations Economic Commission for Europe*. Italy, 13-17 November 1978. Pp. xiii + 416. ISBN-008-025661-9. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$54.00 £25.00.
- WEBB, M. (ed.) *The Chemistry, Biochemistry and Biology of Cadmium. Topics in Environmental Health Volume 2*. Pp. xiii + 465. ISBN-0-444-80109-X. (Amsterdam and New York: Elsevier/North-Holland Biomedical Press, 1979.) US \$80.50 Dfl. 165.00.

- WEISS, D. W. *Tumour Antigenicity and Approaches to Tumour Immunotherapy: Current Topics in Microbiology and Immunology* 89. Pp. 83. ISBN 3-540-09789-9. (Springer: Berlin, Heidelberg and New York, 1980.) DM 29.00; US \$17.20.
- WELCH, E. B. *Ecological Effects of Waste Water: with hydrographic characteristics by T. Lindell*. Pp. 337. ISBN 0-521-22495-0. (Cambridge University Press: Cambridge, 1980.) £15.00; Pbk £5.50.
- WINEFIELD, H. R. and PEAY, M. Y. *Behavioural Science in Medicine*. Pp. xiii + 344. ISBN-0-04-610014-8 hardback ISBN-0-04-610015-6 flexi. (London and Sydney: George Allen & Unwin; Beaconsfield: Beaconsfield Publishers, 1980.) £10.50 hardback £5.95 flexi.
- WALKER, B. H. (ed.) *Management of Semi-Arid Ecosystems. Developments in Agricultural and Managed-Forest Ecology*, 7. Pp. viii + 398. ISBN-0-444-41759-1. (Amsterdam and New York: Elsevier Scientific Publishing Company, 1979.) US \$78.00 Dfl. 160.00.
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- ZUCKERMAN, A. J. and HOWARD, C. R. *Hepatitis Viruses of Man. Experimental Virology Series*. Pp. xi + 269. ISBN-0-12-78150-3. (London, New York, Toronto, Sydney, San Francisco: Academic Press, 1979.) £16.80 \$39.00.

Psychology

- BERKOWITZ, L. (ed.) *Advances in Experimental Social Psychology. Volume 12*. Pp. ix + 358. ISBN-0-12-015122-6. (New York, San Francisco, London: Academic Press, 1979.) \$24.00.
- GRUNBERG, M.M. and MORRIS, P.E. (eds) *Applied Problems in Memory*. Pp. xiii + 289. ISBN-0-12-305150-9. (London, New York, San Francisco: Academic Press, 1979.) £12.40 \$29.00.
- KEEHN, J. D. (ed.) *Origins of Madness. Psychopathology in Animal Life*. Pp. vii + 422. ISBN-0-08-023725-8. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$40.00 £20.00.
- RIEBER, R. W. (ed.) *Language Development and Aphasia in Children: New Essays and a translation of Kindersprache und Aphasie by Emil Fröschels*. Pp. 236. ISBN-0-12-588280-7. (Academic: New York and London, 1980.) \$16.00.
- RUSSELL, B. *Education and the Social Order*. Pp. 158. ISBN-0-04-370080-2. (London, Boston, Sydney: Unwin Paperbacks, 1980.) £1.75 flexi.
- SCHARFETTER, C. *General Psychopathology: An Introduction*. Translated by H. Marshall. Pp. 239. ISBN 0-521-22812-3. (Cambridge University Press: Cambridge, 1980.) £20.00; Pbk £6.95.
- SIMON, H. A. *Models of Thought*. Pp. xviii + 524. ISBN-0-300-02347-2. (New Haven and London: Yale University Press, 1980.) £28.35.
- WILDEN, A. *System and Structure: Essays in Communication and Exchange*. 2nd Ed. Pp. 592. ISBN 0-422-76710-7. (Tavistock London, 1980.) £14.00 Hardback, £6.95 Pbk.

Sociology

- BAYLEY, L. G. *Local Government Is It Manageable? A Critical and Up-to-date Assessment of Local Government Operations with Examples and Explanations*. vii + 125. ISBN-0-08-024279-0. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1980.) \$13.00 £5.50.
- BERTING, J., GEYER, F. and JURKOVICH, R. (eds) *Problems in International Comparative Research in the Social Sciences. Papers from a Symposium on the Theory and Methods of International Comparative Research in the Social Sciences*. Pp. xiii + 180. ISBN-0-08-0252247-8. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1980.) \$22.00 £10.00.
- CARTER, R.L. and HILL, K.Q. *The Criminal's Image of the City. Pergamon Policy Studies on Crime and Justice*. Pp. x + 97. ISBN-0-08-024633-8. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon Press, 1979.) £6.25.
- COOPER, C.L. and PAYNE, R. *Current Concerns in Occupational Stress*. Wiley series on studies in Occupational stress. Pp. x + 341. ISBN-0-471-27624-3. (Chichester, New York, Brisbane, Toronto: John Wiley & Sons, 1980.) £11.75.
- DAMMANN, E. *The Future in Our Hands. What we can all do towards the shaping of a better World. Pergamon International Library of Science, Technology, Engineering and Social Studies*. Pp. xviii + 171. ISBN-0-08-024284-7 hardback ISBN-0-08-024283 flexi. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$20.00 £9.00 Hardback \$10.00 £4.50 flexi.
- GOLDSMITH, M. and KING, A. (eds) *Issues of Development Towards a New Role for Science and Technology: Science, Technology and Global Problems. Proceedings of an International Symposium on Science and Technology for Development, Singapore, January, 1979. Science, Technology and Global Problems Series*. Pp. xxiv + 277. ISBN-0-08-024691-5. (Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979.) \$35.00 £17.50.
- GRIEVES, F. L. (ed.) *Transnationalism in World Politics and Business. Pergamon Policy Studies on U.S. and International Business*. Pp. xiii + 217. ISBN-0-08-023892-0. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon Press, 1979.) £8.75.
- HALE, N.C. *Birth of a Family. The New Role of the Father in Childbirth*. Pp. x + 196. ISBN-0-385-14162-9. (New York: Anchor Press/Doubleday, 1979.) np. Flexi.
- HARRISON SHRYOCK, R. *The Development of Modern Medicine: An Interpretation of the Social and Scientific Factors Involved*. Pp. 473. ISBN 299-07534-6. (University of Wisconsin Press: London and Madison, 1980.) £4.50 Pbk; £10.50.
- MACAULAN, P. *The Ideologies of Planning Law: Urban and Regional Planning Series Vol. 22*. Pp. 282. ISBN 0-08-025198-6 (Pergamon: Oxford, 1980.) Flexi \$20.00; £8.50 Hardback \$40.00; £17.00.
- MORELL, J. A. *Program Evaluation in Social Research. Pergamon International Library*. Pp. xvi + 193. ISBN-0-08-023359-7. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon Press, 1979.) £4.50 flexi.
- O'BRIEN, J.T. and MARCUS, M. (eds) *Crime and Justice in America. Critical issues for the future. Pergamon Policy Studies on Crime and Justice*. Pp. xx + 361. ISBN-0-08-025549-3 Flexi ISBN-0-08-023857-2 Hardback. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon Press, 1979.) Flexi £13.75 Hardback.
- PYKE, M. *Long Life: Expectations for Old Age. Under the Auspices of the British Association for the Advancement of Science*. Pp. 166. ISBN 0-460-04481-8. (J.M. Dent: London, Toronto and Melbourne, 1980.) £6.95; Pbk £3.95.
- SAVITCH, H.V. *Urban Policy and the Exterior City. Federal State and Corporate Impacts upon Major Cities. Pergamon Policy Studies* 41. Pp. xv + 359. ISBN-0-08-023390-2. (New York, Oxford, Toronto, Sydney, Frankfurt, Paris: Pergamon Press, 1979.) £15.00.
- SHRADER-FRECHETTE, K. S. *Nuclear Power and Public Policy: The Social and Ethical Problems of Fission Technology*. Pp. 176. ISBN 90-277-1080-5. (D. Reidel: Dordrecht, Boston and London, 1980.) US \$10.50; Dfl. 20.00 Pbk/Cloth Dfl. 38.00; US \$19.95.

ANNOUNCEMENTS

Awards

The Harvey Prize in Science and Technology for 1980 has been awarded to **Professor Michael O. Rabin** (Hebrew University); the Harvey Prize in Human Health for 1979 is awarded to **Professor Ephraim Racker**, (Cornell University).

The Hamburg Foundation Jung-Stiftung für Wissenschaft und Forschung has awarded the Ernst-Jung-Prize for medicine 1980 to **Sir Alan Parks**, (St Mark's Hospital, London).

The 1st Margaret Grant Memorial Fellowship has been awarded to **E. Shyam Prasad Reddy** (Centre for Cellular and Molecular Biology, Regional Research Laboratory, Hyderabad, India).

The following Junior Beit Fellowships for Medical Research have been awarded: **David John Adams; Ian Brown; Elizabeth Greedy Hill; Elinor Nancy Lupton; Alexandra Marie Thomson.**

The Geological Society of London has made the following awards for 1980: The Wollaston Medal to **Professor Augusto Gansser**; The Murchison Medal to **Professor J.V. Smith**; The Lyell Medal; to **Professor J.R.L. Allen**; The William Smith Medal to **Mr G. Armstrong**; The Wollaston Fund, Murchison Fund and equal moieties of the Lyell Fund to **Drs R.H. Sibson, M.P. Coward, G.S. Boulton and M.R. Leeder**, respectively; The President's Awards to **Drs A.P. Heward, J.K. Leggett and B.W.D. Yardley.**

The Institute of Physics has made the following awards for 1980: Charles Vernon Boys Prize to **Dr A.E. Costley** (National Physical Laboratory, Teddington); Duddell Medal and Prize to **Professor A.V. Crewe** (University of Chicago); Max Born Medal and Prize to **Professor Dr H. Faissner** (Rheinisch-Westfälische Technische Hochschule, Aachen); Glazebrook Medal and Prize to **Mr M.C. Crowley-Milling** (CERN, Geneva); Guthrie Medal and Prize to **Professor M.E. Fisher** (Cornell University); Maxwell Medal and Prize to **Professor D.J. Wallace** (University of Edinburgh); Rutherford Medal and Prize to **Professor P.G. Murphy** (University of Manchester) and **Dr J.J. Thresher** (Rutherford Laboratory, Didcot); Prize for the 1979 Graduateship Examination of the Institute to **Mr D.B. Mackay** (Napier College of Commerce and Technology, Edinburgh); Honorary Fellowship of the Institute to **Professor V.F. Weisskopf** (Massachusetts Institute of Technology).

Person to person

The Bioelectrochemical Society (BES) has been founded with the aim of stimulating cooperation among all scientists interested in the investigation, interpretation and practical utilization of biological phenomena by using electrochemical theories and methods. For information apply to Prof. Dr H.W. Nürnberg, Institute of Chemistry 4 Applied Physical Chemistry, Nuclear Research Center, PO Box 1913, D-5170 Jülich, FRG.

In 1967 a meeting of British research workers in the mucopolysaccharide field resulted in the formation of a Club. One of the first meetings, with the French Connective Tissue Club in Paris lead to the formation of the Federation of European Connective Tissue Societies FECTS which met for the first time in 1968. In September (1980) the Collagen Club and the Mucopolysaccharide Club come together to form the British Connective Tissue Society.

Information on intra-theal overdoses of penicillin is required by J.L. Kay, 14 Ardayre Rd, Prestwick, Scotland. Any factual documented source would be helpful, perhaps reference to toxic effects, such as to the brain and central nervous system, clinical tests, possibly information on animal experiments in related fields and LD 50 tests.

Harvard professor seeks for January 1981 to June 1981 furnished house or flat, preferably in Central London, suitable for two persons plus guest room and study. No children or pets! If exchange is desired: Our 3-bedroomed house is in Lexington (Boston area), 15 min from Harvard, 20 min from MIT, MGH, on 1 acre among trees. Write to Mrs G. Holton, 14 Trotting Horse Drive, Lexington, Massachusetts 02173.

A scientist would like to exchange — for 1 year beginning September 1980 — 3-bedroomed house in an attractive Jerusalem suburb (4 miles from University campus, convenient to University and Medical centres, fully furnished for similar or smaller house or apartment in London. M. Goldberg, Bet Zeit 63, D.N. Harei Yehuda, Israel.

Appointments

Dr J.E. Eastoe is appointed to the Chair of Oral Physiology in the University of Newcastle upon Tyne from 1 October 1980.

Dr David Phillips has been appointed to the new Wolfson Chair of Natural Philosophy in the Royal Institution which was recently endowed by the Wolfson Foundation.

Professor M.H. Williams, currently Professor of Computer Science at Rhodes University, Grahamstown, South Africa, has been appointed to the Chair of Computer Science at Heriot-Watt University, Edinburgh.

Professor Harold W. Woolhouse has been appointed Director of the John Innes Institute, Norwich, and Professor of Biology in the School of Biological Sciences at the University of East Anglia from 1 October 1980.

Dr Paul Freeman has been appointed Director of the Department of Industry's National Engineering Laboratory at East Kilbride to succeed **Mr Denis Mallinson.**

Meetings

16-18 July, **Tropical Animal Production**, Merida (Centre for Tropical Animal Production, Escuela de Medicina Veterinaria, Universidad de Yucatan, Apartado 116D, Merida, Mexico).

21-15 July, **Soil Conservation**, Bedford (Dr R. Morgan, NCAE, Silsoe, Bedford, UK).

23-25 July, **590th Meeting of the Biochemical Society**, Sheffield (The Biochemical Society, 7 Warwick Court, High Holborn, London WC1, UK).

1-4 September, **Environment and Safety Exhibition and Conference**, London (International Environment and Safety Exhibition and Conference, London (International Environment and Safety, Labmate Ltd, Newgate, Sandpit Lane, St Albans, Herts, UK).

3-5 September, **Polymerization Mechanisms**, Liverpool, (Dr John Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

9-11 September, **Industry and Environment**, Loughborough (Administrative Officer, I & E Conference, University of Technology, Loughborough, Leicestershire, UK).

15-18 September, **Oxygen and Life**, Birmingham (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

15-19 September, **Management Studies for Chemists**, Slough (Miss L. Hart, The Royal Society of Chemistry, 30 Russell Square, London WC1, UK).